A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH DACRYOCYSTITIS IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI

In partial fulfilment of the regulations for the award of degree of

M.D. MICROBIOLOGY

(BRANCH – IV)

MADRAS MEDICAL COLLEGE

CHENNAI

APRIL – 2016

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH DACRYOCYSTITIS IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **DR.S.SHANMUGA SUNDARI** during the period of her post graduate study from 2013-2016 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital Chennai – 600003, in partial fulfilment of the requirement of **M.D. MICROBIOLOGY** degree examination of **THE TAMILNADU DR.MGR MEDICAL UNIVERSITY, CHENNAI,** to be held in April 2016.

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DECLARATION

I declare that the dissertation entitled **"A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH DACRYOCYSTITIS IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of **M.D. MICROBIOLOGY** is the record work carried out by me during the period of **October 2014 – August 2015** under the Guidance of **Prof. Dr.Mangala Adisesh, M.D.,** Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai in partial fulfilment of the University regulations for the award of degree of M.D. Microbiology (Branch IV) examination to be held in April 2016.

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ABSTRACT

Introduction:

Inflammation of the lacrimal sac is known as dacryocystitis which usually occurs due to obstruction in the nasolacrimal duct. It is a significant cause of ocular morbidity in children and adults. It can be congenital or acquired. Acquired dacryocystitis can occur as acute and chronic dacryocystitis. Dacryocystitis is mostly caused by bacteria and rarely by fungi. There is a change in etiological agents causing dacryocystitis over the time. Hence this study was undertaken to know the etiological agents of congenital, acute and chronic dacryocystitis.

Aims of the study:

To isolate and identify the bacteria and fungi from clinically diagnosed cases of dacryocystitis. To determine the antimicrobial susceptibility pattern of the bacterial and fungal isolates.

Materials and methods:

A total of 100 clinically diagnosed cases of dacryocystitis of all age groups attending the outpatient department at the Regional Institute of Ophthalmology and Government Ophthalmic Hospital were included in the study. Samples were collected from these patients and processed by standard microbiological techniques.

Results:

Dacryocystitis was more common in the age group of 40 to 50 years.12 patients of congenital dacryocystitis, 8 patients of acute dacryocystitis and 80 patients of chronic dacryocystitis were studied. Females (66%) were more affected than males

(34%). Left eye (63%) was commonly affected than the right eye (35%)and 2% of cases were bilateral. Overall culture positivity was 51.9%. Among the culture positive samples, 90.5% yielded single organism and 9.4% yielded mixed organisms. Totally 58 organisms were isolated, of which 58.6% were Gram positive bacteria, 37.9% were Gram negative bacteria, and 3.4% were fungal isolates.*Streptococcus pneumoniae* was the common pathogen in congenital dacryocystitis and *Staphylococcus aureus* was the common pathogen in acute and chronic dacryocystitis. The incidence of Gram negative pathogens were more in chronic dacryocystitis.Gram positive bacteria were highly sensitive to Gatifloxacin and least sensitive to Ciprofloxacin. Gram negative bacteria were highly sensitive to Tobramycin and least sensitive to Ciprofloxacin and Gentamicin. *Candida albicans* was isolated from two cases of chronic dacryocystitis and both were sensitive to Amphotericin B, Fluconazole, Voriconazole and Itraconazole.

Conclusion:

Microbial culture and sensitivity should be performed in all dacryocystitis cases. This would contribute to the choice of appropriate and effective antimicrobial agents.

Key words: Dacryocystitis, nasolacrimal duct, Staphylococcus aureus.

INTRODUCTION

Inflammation of the lacrimal sac is known as dacryocystitis which usually occurs due to obstruction in the nasolacrimal duct. It has bimodal distribution affecting children less than 1 year and adults over 40 years of age[1]. It is a significant cause of ocular morbidity in children and adults. This disease is more common in patients with poor personal hygiene[2].

Dacryocystitis is an unpleasant disease, as it causes constant watering and discharge. Dacryocystitis is also a threat to the integrity of the eye by becoming the source of infection to orbital cellulitis and panophthalmitis[3,4].

Dacryocystitis can be classified as congenital and acquired dacryocystitis. Acquired dacryocystitis can occur as acute and chronic dacryocystitis. Chronic dacryocystitis is more common[1].

Nasolacrimal duct obstruction(NLD) can occur from different aetiologies, such as primary idiopathic obstruction and secondary obstruction which finally results in stasis of tears, desquamated cells and mucoid secretions in the lacrimal sac, this creates favourable environment for inflammation and infection[5,6].

The lacrimal drainage system is prone for infection due to, contiguity of nasolacrimal duct with conjunctival and nasal mucosal surfaces which are usually colonised with bacteria. In turn dacryocystitis can spread to adjoining structures because of continuity [3].

Dacryocystitis is mostly caused by bacteria and rarely by fungi. The organisms causing dacryocystitis may be different in acute and chronic infections. In chronic dacryocystitis mixed infections are more common[5].

Congenital dacryocystitis is usually presents with epiphora in newborn. Later purulent discharge may develop resulting in matting of eyelashes. When not treated early, complications such as recurrent conjunctivitis, acute on chronic dacryocystitis, lacrimal abscess and fistula formation can occur.

Acute dacryocystitis usually presents with pain and tenderness over the lacrimal sac area. It may present with lacrimal abscess. Complications include acute conjunctivitis, lid abscess, orbital cellulitis, acute ethmoiditis and very rarely cavernous sinus thrombosis.

Chronic dacryocystitis is common than the acute one. It usually presents with persistent watering and discharge from the eye. Complications like chronic conjunctivitis, ectropion of the lower eye lid can occur. Because of prolonged watering, eczema and maceration of lower eye lid skin can occur. It is an important contributory factor for corneal ulcer development and panophthalmitis[7].

In Ophthalmology practice, syringing of the nasolacrimal system is performed preoperative to cataract surgery, in order to exclude dacryocystitis because, it is the risk factor for postoperative infection. If any intraocular surgery is done in the presence of unrecognized dacryocystitis, panophthalmitis can occur[2].

Untreated dacryocystitis will not undergo spontaneous resolution[1].

There are several bacteria known to have been implicated as etiological agents of dacryocystitis. There is a change in etiological agents causing dacryocystitis over the time. So knowing the range of the microorganisms causing dacryocystitis and their antibiotic sensitivity pattern in recent times may help in choosing the appropriate antimicrobial therapy.

There are relatively few studies conducted regarding microbiological characteristics of dacryocystitis. Most of them have studied a specific type of dacryocystitis.

Hence this study was undertaken to know the etiological agents of congenital, acute and chronic dacryocystitis.

AIMS OF THE STUDY

1. To isolate and identify the bacteria and fungi from clinically diagnosed cases of dacryocystitis.
2. To determine the antibiotic susceptibility pattern of the bacterial isolates.
3. To determine the antifungal susceptibility pattern of fungal isolates obtained.
4. To determine and compare the incidence of the various bacterial agents in congenital, acute and chronic cases of dacryocystitis.

REVIEW OF LITERATURE

BACKGROUND:

Dacryocystitis occurs usually due to lacrimal outflow obstruction. It results in stasis of tears in the lacrimal sac. Lacrimal sac infection is usually due to bacterial infections. Only in 1.2% of cases dacryocystitis is caused by fungal organisms (CodenDj[8]).

HISTORICAL PERSPECTIVE:

The disease dacryocystitis has been known from the very earliest times due to its manifestations such as abscesses and fistula on the face. It has been known as Aegilops in the earliest times. The term, Aegilops include all inner canthus swellings. In 1702, George and Sehl of Helle described about Aegilops that, it was not the soft tissue affection but a consequence of obstruction to the lacrimal outflow which results in inflammation**[9]**.

Hirschberg noted that the school of Hippocrates recognized the relationship between epiphora and aging[10]. Lacrimal sac abscess and fistula formation were reported by the ancient Greeks[11].

Platner(1724) observed the presence of nasal disease as a source of infection to dacryocystitis. Later this fact was stressed by Schirmer(1877) and also by Kuhnt(1891-95).

Truce noticed (1900) high incidence of Dacryocystitis among the people in theTropics [12].

The disease commonly occurs in the middle age group, with high incidence in the fifth decade and it is rare in adolescents and children. In adults it is more common in females than males due to the narrow bony canal in females. In the newborn this disease affects both sexes equally. This fact was described by Meller(1929), Ruiz Barrabco, Martinez Romain(1966) and others.

Importance of racial and geographical incidence was described by Santos-Femandez, 1903-21. According to them, this disease is rare in Negroes. It is because of the presence of shorter and wider canal in Negroes.

Kofler (1919), Stenger(1920) and Bockstein(1926) observed that, septal deflection as a cause of nasolacrimal duct obstruction at the lower end. Schaeffer(1920) found that, mucosal abnormalities can produce stasis and also obstruction to the lacrimal outflow.

Dacryocystitis caused by Aspergillosis has been reported by Wright(1930) and Rosen Vold(1942).

Dacryocystitis caused by *Candida albicans* was reported by Fine and Waring(1947). Lacrimal sac abscess due to *Candida albicans* has been reported by Janokta (1970).

One case of bilateral fungal dacryocystitis caused by Candida albicans has been described by Codere F et al in 1982 in a patient who had mid facial trauma [13].

EPIDIMIOLOGY OF DACRYOCYSTITIS:

FREQUENCY:

Individuals having brachycephalic heads will have a higher incidence of dacryocystitis than those having dolichocephalic and mesocephalic heads. This is because; brachycephalic skulls have a narrow diameter of inlet in to the nasolacrimal duct. Individuals having flat nose and narrow face are more prone for development of dacryocystitis. This may be due to the presence of the narrow osseous canal in these individuals[14].

MORBIDITY AND MORTALITY:

In acute dacryocystitis patients will have severe morbidity and rarely mortality. Morbidity is primarily due to lacrimal abscess and spreading of infection.

The morbidity in chronic dacryocystitis is due to chronic watering, matting of eyelashes and inflammation, infection of conjunctiva.

Congenital dacryocystitis is associated with both morbidity and mortality. If not treated properly on time, orbital cellulitis can occur in newborns as orbital septum is poorly formed in them. Other complications like, brain abscess, meningitis and sepsis can occur in congenital dacryocystitis[14, 15].

ANATOMY OF THE LACRIMAL DRAINAGE SYSTEM

The lacrimal apparatus consists of the following structures[16]:

1. Lacrimal gland- secretes tears,
2. Lacrimal punctum,
3. Lacrimal canaliculi,
4. Lacrimal sac,
5. Nasolacrimal duct- carries tears in to nasal cavity.

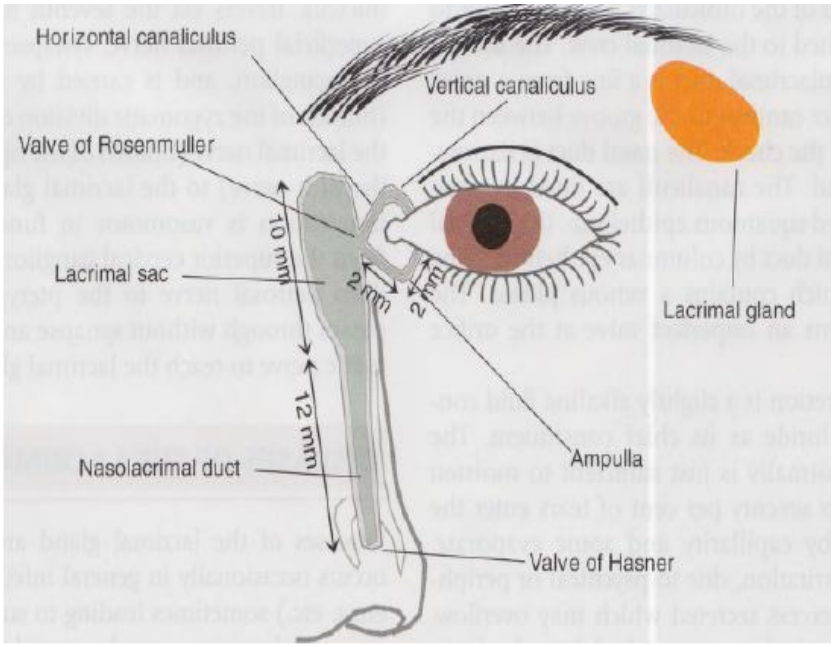


Fig 1: Anatomy of the lacrimal apparatus.

Lacrimal gland:

The lacrimal gland of each eye has an orbital gland, a palpebral gland and accessory lacrimal glands. The orbital gland is situated in the fossa in the orbital roof. Its ducts open upon the conjunctival surface at the upper fornix. The palpebral gland is situated on the course of superior portion ducts. The accessory glands which are microscopic acini, situated below the conjunctival

surface between the fornix and tarsus edge. The ducts of acini, by forming a large duct open in to the fornix.

Lacrimal punctum:

Each eye lid has one punctum. It is situated near the posterior border of the eye lid, 6 mm from the medial canthus.

Lacrimal canaliculi:

Each eye lid has one canaliculi. It starts at the punctum. First it is directed vertically for 2 mm and it turns at the ampulla and then it runs horizontally for 6 to 7 mm. A common canaliculi is formed by the union of upper and lower canaliculi and opens in to the lacrimal sac. A fold of mucosa at this area forms the valve of Rosenmuller. This valve prevents the reflux of tears. The walls of canaliculi are lined by stratified squamous epithelium.

Lacrimal sac:

It is situated in the lacrimal fossa. The lacrimal fossa is formed by frontal process of maxilla and lacrimal bone. The sac is enclosed by lacrimal fascia. The upper part of the sac is the fundus. When distended it is 15 mm long and 5 mm wide. The lower end opens in to the nasolacrimal duct. A fibrous expansion derived from the medial palpebral ligament covers the superficial surface of the lacrimal sac. Lacrimal part of the orbicularis oculi muscle crosses its deep surface. The wall of lacrimal sac is lined by columnar cells.

Nasolacrimal duct:

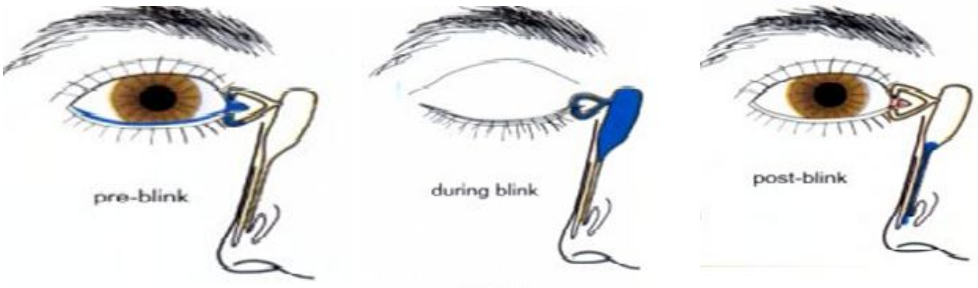
It is 18 mm long. It connects the lower end of lacrimal sac with the inferior turbinate of the nose. In the middle part it is narrower than the both ends. The nasolacrimal duct is lodged in the nasolacrimal bony canal, which lies between the nasal cavity and the maxillary sinus. It runs downwards, slightly outwards and backwards. It opens at the anterior part of inferior meatus of the nose. A mucosal flap forms the valve of Hasner close this opening when necessary. The nasolacrimal duct is lined by columnar cells.

PHYSIOLOGY OF THE LACRIMAL DRAINAGE SYSTEM[1, 17]

Tears secreted by the lacrimal glands pass across the ocular surface, a variable amount of which is lost by evaporation. This is related to blink rate, ambient temperature, humidity and the size of the palpebral aperture. The remaining tears drain as follows(Fig 2):

- Tears flow along the upper and lower marginal strips and it enters by capillary action and suction into the upper and lower canaliculi(Fig 2 A).
- With each blink of eye, the pretarsal orbicularis oculi compresses the ampullae and thereby shortens the horizontal canaliculi and moves the puncta medially. At the same time, the lacrimal part of the orbicularis oculi contracts and compresses the sac, as it is attached to the fascia of the lacrimal sac. This creates a positive pressure which forces tears down the nasolacrimal duct and in to the nose(Fig 2 B &C).

❖ When the eyes open, the muscles relax; the lacrimal sac and canaliculi expand and creates a negative pressure which draws the tears from the eye in to the empty lacrimal sac.



(A) (B) (C)

Fig 2: Physiology of the lacrimal drainage system.

The tear film provides smooth surface for the movement of the eyelids, facilitates the passage of the light, lubricates and provides nutrition to the cornea, and also protects the eyes from injury and infection. Owing to the presence of enzyme lysozyme, the tears have some bacteriostatic properties.The lacrimal secretion has slightly alkaline properties and has sodium chloride as its chief constituents. The normal lacrimal secretion is just sufficient to moisten the eyeball.

DACRYOCYSTITIS

Dacryocystitis is defined as inflammation of the lacrimal sac. It occurs due to obstruction in the nasolacrimal duct. It is an unpleasant disease, because it can cause conspicuous watering and has minimum tendency to resolve. Nasolacrimal duct obstruction can occur from different aetiologies, such as primary idiopathic obstruction or secondary obstruction.

The reasons for secondary obstruction include stricture of the duct caused by chronic atrophic rhinitis, obstruction due to lacrimal sac tumours and maxillary sinusitis; obstruction of the distal end of the duct due to nasal polyp or inferior turbinate hypertrophy. Tuberculosis, syphilis and leprosy-originating from the surrounding bones or nose can also cause secondary obstruction [18].All the above conditions results in stasis of tears, desquamated cells and mucoid secretions in the lacrimal sac, which creates favourable environment for infection. The infection is mostly due to bacteria and rarely due to fungi. Dacryocystitis is treated with topical, systemic antibiotics, probing and silicone stent intubation. Surgery can be done to re-establish the duct patency[19].

Dacryocystitis can occur in two forms, congenital dacryocystitis and acquired dacryocystitis.

CONGENITAL DACRYOCYSTITIS

Etiology:

Congenital dacryocystitis occurs due to congenital blockage of the nasolacrimal duct, which results from incomplete canalization of the

nasolacrimal duct especially at the valve of Hasner. It is also known as dacryocystitis neonatorum [20]. The common bacteria associated with this condition are *Staphylococci*, *Pneumococci* and *Streptococci*[21].

Clinical presentation:

- Epiphora- develops seven days after birth,
- Positive regurgitation test - when pressure is applied over the lacrimal sac area, there may be a mucoid or mucopurulent regurgitation through the puncta,
- Swelling over the sac area may appear.

Complications:

If it is not treated early, complications like,

- Recurrent conjunctivitis,
- Acute on chronic dacryocystitis,
- Lacrimal abscess and formation of fistula can occur[21].

ACQUIRED DACRYOCYSTITIS:

Acquired dacryocystitis can occur both as acute and chronic dacryocystitis. Chronic dacryocystitis is more common.

ACUTE DACRYOCYSTITIS:

Acute dacryocystitis is an acute lacrimal sac inflammation.

Etiology:

It may develop due to direct involvement from infected para nasal sinuses, dental abscess or caries teeth or, as an acute exacerbation of chronic dacryocystitis. The most common organisms responsible are *Staphylococcus aureus* and *Streptococcus species*[22].

Clinical presentation:

It can be divided in to three stages.

1. Stage of cellulitis:

It presents with,

- Painful swelling over the lacrimal sac area,
- Epiphora,
- Fever and malaise,
- Spread of edema to eyelids and cheeks.

2. Stage of lacrimal abscess:

Continuation of inflammation results in occlusion of canaliculi. The lacrimal sac is filled with pus. It presents with large fluctuant swelling pointing below and outer part of the sac.

3. Stage of fistula formation:

If lacrimal abscess is not treated, it spontaneously discharges and external fistula will be formed[21].

Complications:

It includes,

- Mucocele,
- Fistula formation,
- Chronic conjunctivitis,
- Orbital cellulitis.

CHRONIC DACRYOCYSTITIS

Chronic dacryocystitis is more common than the acute one.

Etiology:

The etiological factors are grouped as,

I.Predisposing factors

Age: It is more common over 40 years of age.

Sex: It is more common in females because of comparatively narrow bony canal lumen. As it coincides with menopausal age, an endocrine basis can be suggested. Due to endocrine changes, hypertrophy of the mucous membrane can occur, which will be infected easily and leads to obstruction of nasolacrimal duct[18].

Heredity: It may play a role by affecting the facial configuration with respect to the length and width of the NLD.

Poor personal hygiene: It is another predisposing factor.

II. Factors result in stasis of tears in lacrimal sac

Anatomical factor: Those who are having narrow bony canal are at risk of developing this condition.

Excessive lacrimation: Primary and reflux lacrimation can cause stagnation of tears in the lacrimal sac.

Mild inflammation of sac: It can occur due to recurrent conjunctivitis. The epithelial debris,mucus plugs will block the NLD.

Lower end NLD obstruction: This can occur due to nasal diseases such as, nasal polyps, inferior turbinate hypertrophy, deviated nasal septum, stenosis caused by atrophic rhinitis[21, 23].

III. Source of infection:

Infection can spread from conjunctiva, nasal cavity, paranasal sinuses.

IV. Causative microorganisms:

Staphylococcus species, Pneumococcus species, Pseudomonas species are common infective organisms[21].

Clinical presentation:

It is divided in to four stages.

1.Stage of catarrhal dacryocystitis: The only symptom at this stage is watering from the eye.

2.Stage of lacrimal mucocele: At this stage patients present with constant epiphora, with swelling below the inner canthus region. On

pressing the swelling there will be mucoid discharge regurgitates from the lower punctum.

3.Stage of chronic suppurative dacryocystitis: At this stage, because of pyogenic infections, the mucoid discharge will become purulent. The mucocele is converted to pyocele. Patients present with, epiphora, recurrent conjunctivitis and swelling at the medial canthus. On pressing the swelling there will be frank purulent discharge which regurgitates from the lower punctum.

4.Stage of chronic fibrotic sac: Due to repeated infections, a small fibrotic sac will develop. Patients present with persistent epiphora and discharge.

Complications:

- Chronic conjunctivitis
- Acute on chronic dacryocystitis
- Ectropion of the lower eye lid
- Corneal abrasions, which can be infected and leads to hypopyon corneal ulcer,
- Risk of development of endophthalmitis, if any intraocular surgery is performed in dacryocystitis patients[21].

CLINICAL EVALUATION OF THE NASOLACRIMAL SYSTEM

Examination by diffuse illumination using magnification:

This test is done to rule out causes of reflex hypersecretion located in eye lids, conjunctiva and cornea.

Regurgitation test:

In this test, steady pressure is applied over the lacrimal sac area. Reflux of watery, mucoid or mucopurulent discharge through the punctum indicates obstruction in the nasolacrimal duct.

Syringing[24]:

In this test, after instillation of topical anaesthetic agent, with the help of Nettleship punctum dilator, the lower punctum is dilated. Then saline is injected through a smooth tipped cannula passed into the lacrimal canaliculi. If saline passes into the nose or throat freely, it indicates a patent nasolacrimal duct. In the presence of nasolacrimal duct obstruction, the fluid will regurgitate through the upper punctum.

Fluorescein dye disappearance test[25]:

Apart from syringing it is probably the single most useful test. A drop of 2% fluorescein should be placed in the lower fornix of the both eyes and left for 5 minutes. If the eye remains yellow and the tear meniscus is raised, it is suggestive of abnormality in tear drainage. This test is particularly useful in unilateral epiphora as the normal side goes white and the abnormal side remains yellow.

Nasal endoscopy:

This is helpful in evaluation of diseases of nasal septum and turbinate[14].

Dacryocystography[26]:

This is used to know the level of obstruction. To perform the procedure, a radiopaque material such as lipiodol is pushed into the lacrimal sac by using a lacrimal cannula. Then X rays are taken after 5 and 30 minutes for visualisation of the entire passage.

CT scan:

This is useful in craniofacial injuries, congenital craniofacial deformities. It also helps in evaluation of concomitant sinus or nasal diseases[27].

MANAGEMENT OF CONGENITAL DACRYOCYSTITIS[21]:

- **Up to 6-8 weeks of age:**

  Massage over the lacrimal sac area should be done at least 4 times per day. This should be followed by installation of antibiotic drops. This method can cure 90% of infants.

- **Up to 2 months of age:**

  If the condition is not cured by the above management, lacrimal syringing with normal saline can be done. It should be followed by

antibiotic installation. Syringing should be carried out once or twice a week.

- **Up to 3-4 months of age:**

  If the condition is not cured by syringing, Probing of nasolacrimal duct with Bowman's probe can be done. It is performed under general anaesthesia. If it is failed the same procedure can be repeated after one month.

- **Up to 4 years:**

  Conservative treatment like, massaging, topical antibiotics and intermittent syringing should be done up to 4 years of age.

- **After 4 years of age:**

  Dacryocystorhinostomy surgery can be performed.

MANAGEMENT OF ACUTE DACRYOCYSTITIS:

At the Stage of cellulitis:

At this stage, treatment consists of systemic antibiotics and anti-inflammatory drugs with hot fomentation.

At the Stage of lacrimal abscess:

At this stage, in addition to the antibiotics, incision & drainage should be performed. Later, to prevent recurrence, either Dacryocystectomy or Dacryocystorhinostomy surgery can be performed, depending on the lacrimal sac condition.

At the Stage of fistula formation:

Fistulectomy along with Dacryocystectomy or Dacryocystorhinostomy can be done.

MANAGEMENT OF CHRONIC DACRYOCYSTITIS:

Conservative treatment:

In the early period, repeated lacrimal syringing can be done. Antibiotics should be given.

Surgical procedures:

1. Dacryocystorhinostomy (DCR):

It is the preferred surgery because the lacrimal drainage is reestablished. The infection should be controlled before doing the surgery. DCR can be performed either by conventional external approach or by endo nasal DCR.

2.Dacryocystectomy (DCT):

When DCR is contraindicated, this surgery can be performed. Indications are,

- Patients more than 60 years of age,
- Presence of shrunken and fibrosed sac,
- Infections with tuberculosis, leprosy and mycotic infections
- Lacrimal sac tumours
- Nasal diseases particularly atrophic rhinitis

3.Conjunctivodacryocystorhinostomy:

This surgery is done, if blocked canaliculi are present[21].

MICROBIOLOGY OF DACRYOCYSTITIS

Normal flora of the conjunctiva:

The predominant organisms of the conjunctiva include, *diphtheroids* mainly *Corynebacterium xerosis, Moraxella species, Staphylococcus epidermidis* and *nonhemolytic Streptococci*. Normally the conjunctival flora is held in check by the flow of tears and the antibacterial lysozyme it contain[28,29].

Bacterial and fungal profile of dacryocystitis:

Because of the contiguity of mucous lined tract of the lacrimal drainage system with conjunctival and nasal mucosal surfaces, organisms from both ends of lacrimal passage can infect the lacrimal sac. Infection from para nasal sinuses and oral cavity can also spread to the sac.

The common bacteria causing dacryocystitis are *Streptococcus pneumoniae, Staphylococcus aureus, Streptococcus pyogenes* and *Haemophilus influenzae*. The common fungi causing dacryocystitis are *Candida albicans, Aspergillus species*[30].

Syed Ali Raza Rizvi et al [31] in March 2015 studied 86 patients who presented with acute and chronic dacryocystitis. It included 23 male patients and 63 female patients. Ratio of male to female was 1:3. Percentage of culture positivity was 76.47% in acute cases and 37.84% in chronic cases. In acute cases the predominant organism was *Streptococcus pneumoniae* (53.8%) followed by *Staphylococcus aureus* (46.1%). No gram negative bacteria were

isolated in acute cases. In chronic cases also the predominant organism was *Streptococcus pneumoniae*(35.7%) followed by *Staphylococcus aureus*(28.5%)and *Pseudomonas aeruginosa*(25%).

JithendraKandati et al[5] in January 2015 studied 298 patients of dacryocystitis. It included 126 acute cases and 172 chronic cases. In both acute and chronic cases, females were more affected than males. Bacterial and fungal culture was performed in all the cases. Culture positivity was 85.57%. In two cases of chronic dacryocystitis, *Candida albicans* was isolated. Polymicrobial growth was exclusively present in chronic dacryocystitis. Overall, *Staphylococcus aureus*(24.5%) was the common gram positive bacteria isolated, followed by *Staphylococcus epidermidis*(20.3%) and *Streptococcus pneumoniae*(3.8%). *Pseudomonas aeruginosa*(21.3%) was the common gram negative bacteria isolated, followed by *Escherichia coli*(16.8%), *Klebsiella pneumoniae* (7%).

IndrajitSarkar et al[32] in July 2015 studied 90 patients of dacryocystitis. It included 3 cases of congenital cases and 87 cases of chronic cases. Culture positivity was 83.33%. Single organism was isolated in 73.3% of samples, mixed organisms were isolated in 10% of samples.78.57%of isolates were Gram-positive bacteria and 21.43% of isolates were Gram-negative bacteria. The predominant organism isolated was *Staphylococcus aureus*(57.1%) followed by *Streptococcus pneumoniae*(14.2%), *Pseudomonas aeruginosa*(13%) and *Klebsiella pneumoniae* (7.1%).

Shwetha B A et al[3] in 2014 studied 200 patients of chronic dacryocystitis. The percentage of culture positivity was 57.5%. The predominant organism isolated was *Staphylococcus aureus*(32.17%) followed by *Klebsiella pneumoniae* (14.78%),*Streptococcus pneumoniae*(11.3%), *Staphylococcus epidermidis*(8.69%) and mixed growth (6.08%).

Bahram Eshraghi et al **[33]** in 2014 studied 100 patients of dacryocystitis which included 60 acute cases and 40 chronic cases. Overall culture positivity was 85%. *Staphylococcus aureus* was the predominant organism isolated from acute cases (35%) followed by *Streptococcus viridans* (17%), *Klebsiella species*(8%), *Citrobacter species*(8%), *Pseudomonas aeruginosa*(4%), *Escherichia coli*(4%),*Staphylococcus epidermidis*(4%) and *Aspergillus species*(2%).In chronic cases *Staphylococcus epidermidis* (38%) was the predominant organism isolated, followed by *Streptococcus viridans*(20%), *Klebsiella species*(10%) and *Staphylococcus aureus*(10%).

Khevna Patel et al **[2]** in 2014 studied 100 patients of chronic dacryocystitis which included 52 female patients and 48 male patients. Culture positivity was seen in 83% of samples. The predominant organism isolated was *Staphylococcus aureus*(41%) followed by *Escherichia coli*(17%), *Pseudomonas aeruginosa*(12%), *Streptococcus pneumoniae*(9%) and *Klebsiella pneumoniae*(3%).

Pradeep A.V et al**[34]** in 2013 studied 44 patients of chronic dacryocystitis. Lacrimal sac contents obtained during external dacryocystorhinostomy procedure were processed. A total of 21 organisms

were isolated from 44 specimens. The predominant isolate was *Coagulase negative Staphylococci*(71%), followed by *Staphylococcus aureus* (14%), *Escherichia coli*(4.7%) and *Citrobacter freundii*(4.7%).

Prakash R et al[35]in 2012 studied 80 patients of dacryocystitis which included both congenital and acquired cases. Females (70%) were more affected than males(30%). *Staphylococcus aureus* (29.76%) was the common organism isolated in acquired cases, followed by *Streptococcus pneumoniae*(20.23%), *Pseudomonas aeuginosa*(14.28%), *Klebsiella pneumoniae*(8.33%) and *Staphylococcus epidermidis*(8.33%). *Streptococcus pneumoniae*(50%) was the common organism isolated in congenital cases, followed by *Pseudomonas aeruginosa*(20%) and *Staphylococcus aureus*(10%).

Shah C P et al[36] in 2011 studied 100 cases of dacryocystitis, which included 45 acute cases and 55 chronic cases. Culture positivity was 100%. Mixed cultures were isolated in 9.1% of samples. *Escherichia coli* was the predominant organism isolated from acute cases (22.9%) followed by *Staphylococcus aureus*(18.75%), *Pseudomonas aeruginosa*(18.75%), *Coagulase negative Staphylococci*(16.6%), *Streptococcus species*(12.5%) and *Klebsiella species*(10.4%). *Staphylococcus aureus* was the predominant organism isolated from chronic cases (27%) followed by, *Pseudomonas aeruginosa*(21.6%), *Klebsiella species*(14.8%), *Coagulase negative Staphylococci*(12.1%), *Streptococci species*(12.1%) and *Escherichia coli*(12.1%).

Chaudhary M et al [37] in 2010 studied 120 patients of chronic dacryocystitis. Culture positivity was 76.66%. Single organism was isolated in 85.86% and mixed growth was present in 14.13% of culture positive cases. The predominant organism isolated was *Coagulase Negative Staphylococci*(33.96%) followed by, *Staphylococcus aureus*(25.46%) and *Streptococcus pneumoniae*(19.81%). *Escherichia coli*(5.66%) was the common gram negative organism isolated.

Bharathi M J et al[38] in 2008 studied 1891 patients of dacryocystitis, which included 566 patients of acute dacryocystitis, and 1325 patients of chronic dacryocystitis. Culture was positive from 80.3% of the samples. The percentage of culture positivity was 90% in chronic dacryocystitis and 57.4% in acute dacryocystitis. In acute dacryocystitis the predominant organism was *Staphylococcus aureus*(22.3%) followed by *Pseudomonas aeruginosa*(21.1%). In chronic dacryocystitis the predominant organism was *Coagulase negative Staphylococci*(44.2%) followed by *Staphylococcus aureus*(10.8%) and *Streptococcus pneumoniae*(10%).

Mandal R et al [39] in 2008 studied 56 patients of chronic dacryocystitis who underwent either dacryocystectomy or dacryocystorhinostomy. Part of the sac was collected as samples. Culture positivity was 53.6%. *Staphylococcus aureus*(40%) was the common gram positive organism isolated, followed by *Staphylococcus epidermidis*(10%) and *Streptococcus pneumoniae*(10%). *Pseudomonas aeruginosa* (16.6%) was the common gram negative organism

isolated, followed by *Klebsiella pneumoniae*(6.6%) and *Haemophilus influenzae* (6.6%).

BhavnaRaina et al [40] in 2010 studied 30 cases of congenital dacryocystitis. Culture positivity was 72.9%. Majority of bacterial isolates were Gram positive cocci (56.7%). *Streptococcus pneumoniae* (27.9%) was the predominant organism isolated, followed by *Staphylococcus aureus*(16.2%) and *Staphylococcus albus*(10.8%).

Andreas Kuchar et al [41] in 2000 studied 47 cases of congenital dacryocystitis, which included 3 bilateral cases. Totally 50 samples were studied. Culture positivity was 72.64%. 20 samples had mixed growth. The most common organism isolated was *Streptococcus pneumoniae*(35.4%), followed by *Haemophillus influenzae* (19.6%), *Pseudomonas aeruginosa*(10.9%) and *Streptococcus viridans*(10.9%).

MATERIALS AND METHODS

This study was carried out in the Institute of Microbiology, Madras Medical College, Chennai in association with Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Chennai. A total of 100 clinically diagnosed cases of dacryocystitis of all age groups attending outpatient department at the Regional Institute of Ophthalmology and Government Ophthalmic Hospital were included in the study. The patients were categorized as acute, chronic and congenital dacryocystitis based on the age of onset, symptoms and signs.

ETHICAL CLEARANCE:

Before the commencement of the study, approval was obtained from the Institutional Ethics Committee. Informed consent was obtained from all the patients who satisfied the inclusion criteria.

STUDY DESIGN:

Cross sectional study.

STUDY PERIOD:

October 2014 to August 2015

INCLUSION CRITERIA:

Clinically diagnosed cases of dacryocystitis of all age groups were included in the study and categorised as follows.

- Patients presenting with pain, redness and swelling in the region of lacrimal sac were included as acute dacryocystitis.
- Adult patients presenting with persistent epiphora for more than 3 weeks and regurgitation of serous, mucoid or mucopurulent material on pressure over the lacrimal sac area or on lacrimal syringing were included as chronic dacryocystitis.
- Children presenting with persistent epiphora from the first week of birth and regurgitation of serous, mucoid or mucopurulent material on pressure over the lacrimal sac area or on lacrimal syringing were included as congenital dacryocystitis.

EXCLUSION CRITERIA:

- The patients with the above symptoms who had received either topical or systemic antibiotics for the past one week during their visit to the hospital were excluded from the study.
- All cases of pseudo epiphora and epiphora caused by conditions other than nasolacrimal duct obstruction were excluded from the study.

The diagnosis of dacryocystitis when given by Ophthalmologist, then samples were collected with their help.

SAMPLE COLLECTION:

A salient history was obtained from the patients before the collection of samples. Under strict aseptic precautions, samples were collected in three sterile swabs.

SAMPLES:

- Pus or mucopurulent discharge drained by incision and drainage was collected in cases of acute dacryocystitis.
- Serous, mucoid or mucopurulent discharge obtained by syringing of the lacrimal sac was collected in cases of chronic dacryocystitis. Lacrimal sac contents obtained during DCT or DCR were also collected in few cases.
- Serous, mucoid or mucopurulent discharge obtained by syringing of the lacrimal sac or obtained on pressure over the lacrimal sac area was collected in cases of congenital dacryocystitis. Lacrimal sac contents obtained during DCR were also collected in some cases.

TRANSPORTATION AND PROCESSING OF SAMPLES:

The samples were transported to the laboratory immediately and processed. Of the three swabs collected, one swab was used for direct gram staining and KOH mount. The other swab was used for inoculation of bacterial culture. The last swab was used for inoculation of fungal culture.

DIRECT EXAMINATION:

Direct gram staining:

The smear was prepared on a clean grease free glass slide. After air drying heat fixation was done. Gram staining was performed. The stained smear was screened for pus cells and bacteria, their gram reaction,

arrangement, morphology and presence of gram positive budding yeast cells if any.

10% KOH wet mount:

The sample was added to a drop of 10% KOH on a clean glass slide. A coverslip was placed over the drop and allowed to stand at room temperature for 10 minutes and examined under microscope using low power and high power objectives for the presence of fungal elements.

BACTERIAL CULTURE:

The next swab was inoculated on to MacConkey agar plate, Blood agar plate and were incubated at 37ºC and on to chocolate agar plate and incubated at 5-10% CO_2 atmosphere at 37°C for 24 hours.

After 24 hours of incubation the culture plates were observed for growth, morphology of colonies and were subjected to Gram staining. If Gram staining shows Gram positive cocci, catalase test, coagulase test and other standard biochemical reactions and tests[42, 43] were done. If Gram staining shows Gram negative bacilli, catalase test, oxidase test, motility by hanging drop method and other standard biochemical reactions and tests were done.

If there was no growth at 24 hours, the plates were further incubated for another 24 hours. If no growth was observed after 48 hours of incubation the culture was considered as negative for aerobic bacterial growth.

Bacterial cultures were considered significant, if

- Growth in the media was consistent with findings of direct microscopy,

- Presence of confluent growth at the inoculation site,
- Same growth of organism was demonstrated on all the inoculated culture media plates.

IDENTIFICATION OF ISOLATES:

- Beta hemolytic colonies and golden yellow pigment on blood agar, Gram positive cocci in clusters in Gram stain, catalase test positive, slide coagulase test positive, tube coagulase test positive, urease test positive, mannitol fermenting, phosphatase producing, MR test positive and VP test positive isolates were identified as *Staphylococcus aureus.*
- White opaque colonies on blood agar, Gram positive cocci in clusters in Gram stain, catalase test positive, slide coagulase test negative, tube coagulase test negative, phosphatase producing, Novobiocin sensitive and Polymyxin B resistant isolates were identified as *Staphylococcus epidermidis.*
- Alpha haemolytic colonies on blood agar, Gram positive flame shaped diplococci in Gram stain, catalase test negative, optochin sensitive, bile solubility test positive and inulin fermenting isolates were identified as *Streptococcus pneumoniae*.
- Non-haemolytic tiny colonies on blood agar, Gram positive cocci in pairs and short chains in Gram stain, catalase test negative, bile esculin test positive, arginine dihydrolase test positive, heat tolerant (surviving at 60^{o}C for 30 min), mannitol fermenting, arabinose non-fermenting isolates were identified as *Enterococcus faecalis.*

- Lactose fermenting mucoid colonies on MacConkey agar, short Gram negative bacilli in Gram stain, non-motile bacilli detected by hanging drop method, catalase test positive, oxidase test negative, nitrate reduction test positive, indole test negative, MR test negative, VP test positive, citrate utilization test positive, acid butt and acid slant with gas on TSI, lysine decarboxylase test positive, ornithine decarboxylase test negative, urease test positive isolates were identified as *Klebsiella pneumoniae*.
- Lactose fermenting colonies on MacConkey agar, Gram negative bacilli in Gram stain, motile bacilli detected by hanging drop method, catalase test positive, oxidase test negative, nitrate reduction test positive, indole test positive, MR test positive, VP test negative, citrate utilization test negative, acid butt and acid slant with gas on TSI, lysine decarboxylase test positive,urease test negative isolates were identified as *Escherichia coli.*
- Non-lactose fermenting colonies on MacConkey agar, bluish green pigment producing colonies on nutrient agar, slender Gram negative bacilli in Gram stain, motile bacilli detected by hanging drop method, catalase test positive, oxidase test positive, oxidative reaction in Huge &Leifson O/F medium, nitrate reduction test positive, MR test negative, VP test negative, alkaline butt and alkaline slant on TSI, arginine dihydrolase test positive and lysine decarboxylase test negative isolates were identified as *Pseudomonas aeruginosa*.

ANTIMICROBIAL SUSCEPTIBILITY TESTING:[44,46]

All the bacterial isolates obtained were subjected to antimicrobial susceptibility testing by using Kirby-Bauer disc diffusion method. To know the efficacy, antibiotic discs were tested against standard American Type Culture Collection (ATCC) control strains, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 as a quality control laboratory procedure.

Antimicrobial susceptibility testing by Kirby – Bauer Disc Diffusion method:

- 3- 5 well isolated colonies from an agar plate culture were touched by a sterile bacteriological loop and emulsified in 3-4ml of sterile peptone water. The bacterial suspension was matched to a 0.5 McFarland standards.
- By using a sterile cotton swab, the suspension was streaked in three directions on to the surface of a Mueller Hinton Agar plate. Mueller Hinton Agar with 5% sheep blood was used for antibiotic sensitivity testing of *Streptococcus pneumoniae.*
- With the lid in place, the inoculated agar surface was allowed to dry for 3 to 5 minutes before placing the antibiotic discs.
- By using sterile forceps, appropriate antibiotic discs were placed on the surface of the agar. Five discs were placed per 90 mm plate.

The plates were incubated overnight at 37^{o}C. After overnight incubation, the diameter of zone of inhibition was measured and interpreted as sensitive or resistant according to the CLSI guidelines[44].

Panel of antibiotics used in antimicrobial susceptibility testing of Gram positive cocci.

Antibiotic	Disc content	Gram positive cocci	Diameter of zone of inhibition in mm[44]		
			Sensitive	Inter - mediate	Resistant
Penicillin	10 units	*Staphylococcus species*	≥ 29	-	≤ 28
Cefoxitin	30 µg	*Staphylococcus aureus*	≥ 22	-	≤ 21
		CoNS	≥ 25	-	≤ 24
Oxacillin	1 µg	*S.pneumoniae*	≥20		
Amikacin	30 µg	*Staphylococcus species*	≥ 17	15-16	≤ 14
Tobramycin	10 µg	*Staphylococcus species*	≥15	13-14	≤12
Erythromycin	15 µg	*Staphylococcus species& Enterococcus species*	≥ 23	14-22	≤13
		S.pneumoniae	≥ 21	16-20	≤15
Ciprofloxacin	5 µg	*Staphylococcus species& Enterococcus species*	≥ 21	16-20	≤15
Gatifloxacin	5 µg	*Staphylococcus species*	≥ 23	20-22	≤19
		S.pneumoniae	≥ 21	18-20	≤17
Trimethoprim-Sulfamethoxazole	1.25/ 23.75µg	*S.pneumoniae*	≥ 19	16-18	≤15
Ampicillin	10 µg	*Enterococcus species*	≥17	-	≤16
Vancomycin	30 µg	*Enterococcus species*	≥17	15-16	≤14
Tetracycline	30 µg	*Enterococcus species& Staphylococcus species*	≥ 19	15-18	≤14
		S.pneumoniae	≥ 28	25-27	≤24
Chloramphenicol	30 µg	*Staphylococcus species& Enterococcus species*	≥ 18	13-17	≤12
		S.pneumoniae	≥ 21	-	≤20

Panel of antibiotics used in antimicrobial susceptibility testing of Gram negative bacilli.

Antibiotic	Disc content	Gram negative bacilli	Diameter of zone of inhibition in mm[44]		
			Sensitive	Inter - mediate	Resistant
Amikacin	30 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥ 17	15-16	≤ 14
Gentamicin	10 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥15	13-14	≤12
Cefotaxime	30µg	*Enterobacteriaceae*	≥26	23-25	≤22
Ceftazidime	30µg	*Enterobacteriaceae*	≥21	18-20	≤17
		Pseudomonas aeruginosa	≥18	15-17	≤14
Ciprofloxacin	5 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥ 21	16-20	≤15
Tobramycin	10 µg	*Enterobacteriaceae &Pseudomonas aeruginosa*	≥15	13-14	≤12
Gatifloxacin	5 µg	*Enterobacteriaceae*	≥18	15-17	≤14
Chloramphenicol	30 µg	*Enterobacteriaceae*	≥ 18	13-17	≤12
Piperacillin-tazobactam	100µg/ 10 µg	*Pseudomonas aeruginosa*	≥21	15-20	≤14
Tetracycline	30 µg	*Enterobacteriaceae*	≥15	12-14	≤11

Detection of methicillin resistance in *Staphylococcus aureus* and *Coagulase negative Staphylococci (CoNS)* isolates by cefoxitin disc diffusion test [44]:

All *Staphylococcus aureus* and *Coagulase negative Staphylococci (CoNS)* isolates were subjected to cefoxitin disc diffusion test. Cefoxitin is used as a surrogate marker for detection of *mecA*-mediated oxacillin resistance [44].The bacterial suspension of test isolates were matched to a 0.5 McFarland standards and lawn cultured on Mueller Hinton Agar plates separately. Cefoxitin (30µg) disc were placed on the surface of lawn culture of the isolates. The plates were incubated in ambient air at 35°C for 24 hours.

Interpretation as per CLSI guidelines:

For *Staphylococcus aureus and Staphylococcus lugdunensis*

Zone of inhibition ≥22mm - Sensitive

Zone of inhibition ≤21mm - Resistant

For *Coagulase negative Staphylococci* except *Staphylococcus lugdunensis*

Zone of inhibition ≥25mm - Sensitive

Zone of inhibition ≤24mm - Resistant

Quality control strain used - *Staphylococcus aureus* ATCC 25923.

Screening Test for Extended -Spectrum β-Lactamases (ESBLs) in *Klebsiella pneumoniae* and *Escherichia coli* isolates[44,45].

All the *Klebsiella pneumoniae* and *Escherichia coli* isolates were subjected to initial screening test for Extended -Spectrum β-Lactamases by using Cefotaxime and Ceftazidime discs as per CLSI guidelines. The use of more than one antimicrobial agent for screening improves the sensitivity of ESBL detection [44].

Test method - Disk diffusion

Procedure:

The bacterial suspension of test isolates were matched to a 0.5 McFarland standards and lawn cultured on Mueller Hinton Agar plates separately. Ceftazidime (30 µg) and Cefotaxime (30 µg) discs were placed on the surface of lawn culture of the isolates. The plates were incubated in ambient air at 37°C for 16-18 hours.

Results:

Ceftazidime zone ≤22 mm, Cefotaxime zone ≤27 mm may indicate ESBL production.

FUNGAL CULTURE:

The last swab was inoculated on to two Sabourauds Dextrose Agar (SDA) slopes and incubated at 25^0C and 37^0C. The culture was examined daily for one week and then three times per week for the next three weeks. If there is no growth at 4 weeks of incubation, the culture was considered as negative for fungal growth.

IDENTIFICATION OF FUNGAL ISOLATES[47]:

If growth was seen on SDA slope, the morphology of colonies was observed. If cream coloured pasty colonies were seen, Gram staining was performed. Presence of Gram positive ovoid budding yeast cells in Gram staining was suggestive of *Candida species.* The following tests were done.

Germ tube test:

0.5 ml of sterile serum was taken in a sterile test tube. With a sterile bacteriological loop, a colony of yeast was touched and emulsified in the serum. The tube was incubated at 37^oC for 2 hours. After 2 hours, a drop of serum was transferred to a clean glass slide. A coverslip was put and examined under microscope using low power and high power objectives[47,48].

Interpretation:

The test was considered as positive if there is formation of germ tube and there is no constriction between the yeast cell and the germination tube.

Candida CHROM Agar:

The yeast colonies were inoculated on to Candida CHROM Agar and incubated at 37^{o}C. The plates were read after 48 hours and the colour of the colony was observed.

Observation & Interpretation:

Candida albicans	-	Light green
Candida dubliniensis	-	Dark green
Candida glabrata	-	Pink to Purple
Candida krusei	-	Pink
Candida parapsilosis	-	Cream to pale pink
Candida tropicalis	-	Blue with pink hallow[49]

Corn Meal Tween 80 Agar (Dalmau Plate Culture Technique)[48,49]

- By using a straight wire an isolated colony from the primary culture media was taken and inoculated on to cornmeal agar plate by making three parallel lines.
- A sterile cover slip was placed over the surface of the agar, covering the inoculated streaks in such a way that the streak project beyond the coverslip.
- The plate was incubated at 25^{0}C for 48 hours.

- After 48 hours, the edge of the coverslip was examined under low power objective and high power objective for the presence of pseudo hyphae, blastoconidia and chlamydospores.

ANTIFUNGAL SUSCEPTIBILITY TESTING OF YEAST:

- Disc Diffusion method
- Broth Micro dilution Method

Disc diffusion method[50]:

This method was carried out following the M 44-A CLSI guidelines using fluconazole and voriconazole discs.

Inoculum preparation and application of discs:

- With a sterile bacteriological loop, 3- 5 yeast colonies were taken from the culture grown on SDA and emulsified in 5ml of sterile saline.
- The yeast suspension was matched to a 0.5 McFarland standards.
- By using a sterile cotton swab, the suspension was streaked in three directions on to the surface of a Mueller Hinton Agar plate supplemented with 2% glucose and 0.5 µg/ml methylene blue.
- By using sterile forceps, fluconazole and voriconazole discs were placed on the surface of the agar.

The plates were incubated at 37°C. After 24 hours of incubation, the diameter of zone of inhibition was measured and interpreted as sensitive or resistant according to the CLSI guidelines[44A]. Quality control strain used-ATCC Candida albicans 90028.

Antifungal disc	Disc content	Diameter of zone of inhibition in mm		
		Sensitive	Susceptible Dose Dependent	Resistant
Fluconazole	25 µg	≥17	14-16	≤13
Voriconazole	1 µg	≥17	14-16	≤13

Broth micro dilution method[51]

The test was performed as per the CLSI guidelines. Minimal Inhibitory Concentration of Fluconazole, Amphotericin B and Itraconazole were determined. Dimethyl sulfoxide (DMSO) was used as solvent. Media used was RPMI 1640.Preparation of inoculum and antifungal stock solution was done according to the CLSI guidelines.

The concentrations of the drugs tested were,

Amphotericin B 16 to 0.0313µg/ml

Fluconazole 64 to 0.125 µg/ml

Itraconazole 16 to 0.0313 µg/ml

Quality control strain used-ATCC Candida albicans 90028.

Procedure:

- The broth micro dilution test was performed by using sterile, disposable, multi well micro dilution plates (96U-shaped wells).
- 100µl of varying drug concentrations was dispensed in each row from 1 to 10. Then 100µl of test inoculum was dispensed from row 1 to 10.
- Row 11 was the growth control well contains 100 µl of sterile, drug-free medium and was inoculated with 100 µl of the corresponding inoculum suspension. Row 12 was the drug control well contains 100 µl of drug and 100 µl of sterile, drug-free medium.
- The micro dilution plates were incubated at 35^0C for 48 hours. After 48 hours the micro dilution wells were observed with the aid of reading mirror. The growth in each well was compared with that of the growth control well.
- For Amphotericin B, MIC was interpreted as the lowest concentration in which the well was clear.
- For Fluconazole and Itraconazole, MIC was interpreted as the lowest concentration in which a prominent decrease in turbidity was observed. A prominent decrease in turbidity corresponds to approximately 50% inhibition in growth as determined spectrophotometrically.

Interpretive Guidelines for In Vitro Susceptibility Testing of *Candida* Species:

Break points (µg/ml) for Candida species.

Antifungal agent	Susceptible	Susceptible Dose Dependent	Resistant
Fluconazole	≤ 8	16-32	≥64
Itraconazole	≤ 0.125	0.25-0.5	≥1
Amphotericin B	≤ 1	-	>1

Statistical analysis:

Statistical analysis was done by using Statistical Package for Social Sciences (SPSS) version 20.0. Pearson Chi-square(X^2) statistics was carried out, P value <0.05 is considered as statistically significant.

RESULTS & ANALYSIS

This study was carried out at the Institute of Microbiology, Madras Medical College, Chennai, in association with Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Chennai. 100 clinically diagnosed patients of dacryocystitis of all age groups were studied from October 2014 to August 2015.

Out of 100 patients, 98 patients had unilateral dacryocystitis and 2 patients had bilateral dacryocystitis. Totally 102 samples were collected from 100 patients.

TABLE NO: 1. ANALYSIS OF AGE DISTRIBUTION OF DACRYOCYSTITIS CASES

Age group in years	Male	Female	Total	
			No	Percentage
Below 10	7	5	12	12 %
11-20	0	1	1	1 %
21-30	0	2	2	2 %
31-40	3	6	9	9 %
41-50	4	23	27	27 %
51-60	8	14	22	22 %
61-70	7	11	18	18 %
71-80	5	4	9	9 %

This study shows highest number of acquired dacryocystitis cases among people in the age group of 41-50 years(27%) followed by 51-60 years (22%)and 61-70 years(18%). Overall the mean age is 47.97 years. The highest number of females were in the age group of 41-50 years and of males were in the age group of 51-60 years.

Distribution of dacryocystitis cases according to age.

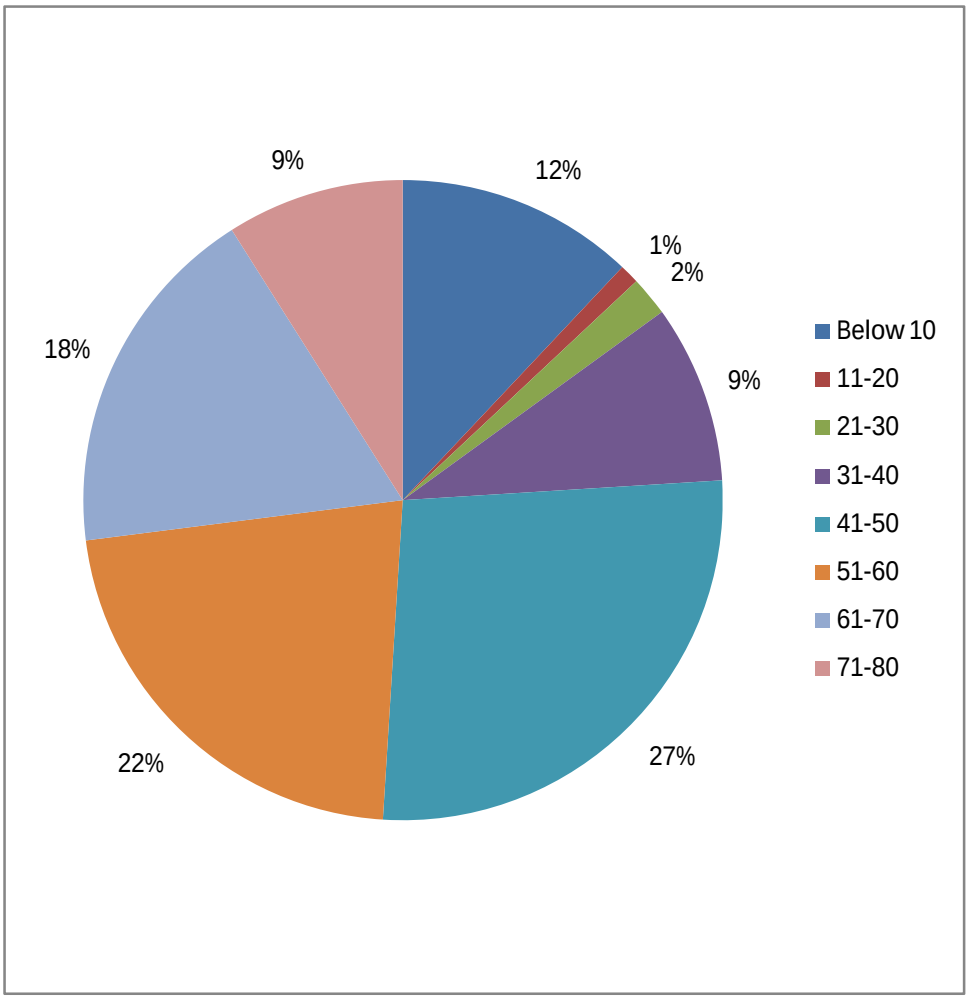


TABLE NO: 2. CLINICAL DIAGNOSIS OF DACRYOCYSTITIS AND THEIR DISTRIBUTION

Clinical diagnosis of dacryocystitis	Total no of cases	Percentage	
Congenital dacryocystitis	12	12%	P value<0.05
Acute dacryocystitis	8	8%	
Chronic dacryocystitis	80	80%	

From the above table it is observed that, in our study chronic dacryocystitis is more common than the congenital and acute dacryocystitis and it is statistically significant.

Clinical diagnosis of dacryocystitis and their distribution

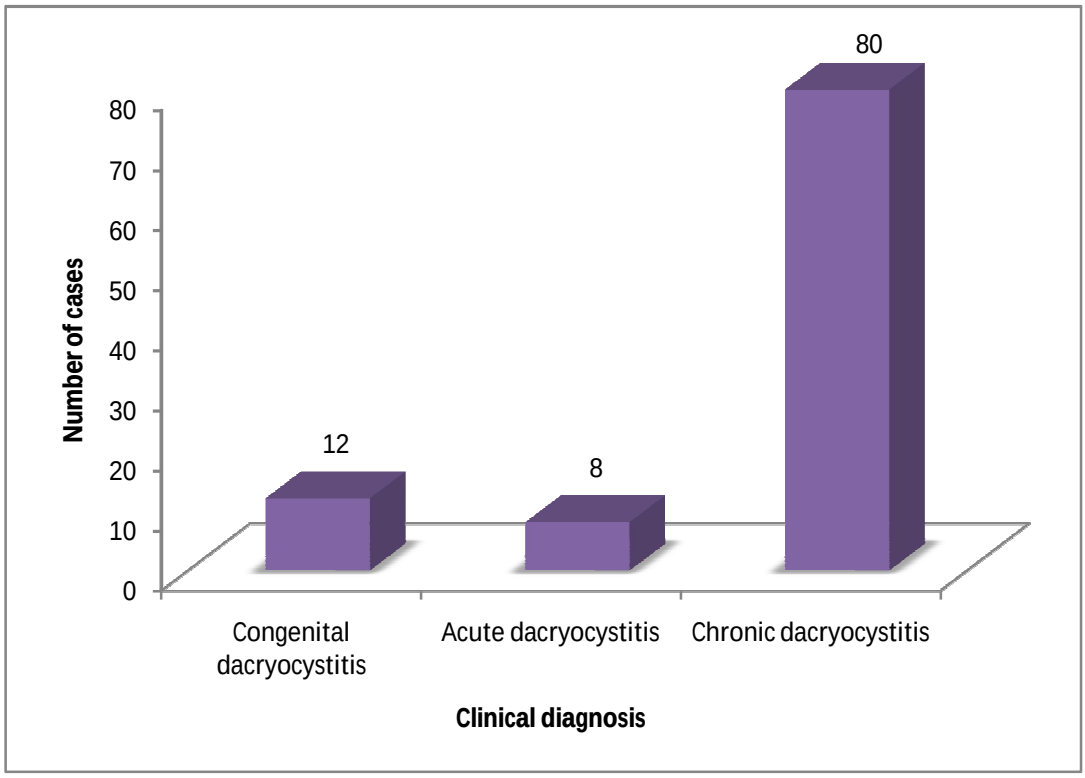
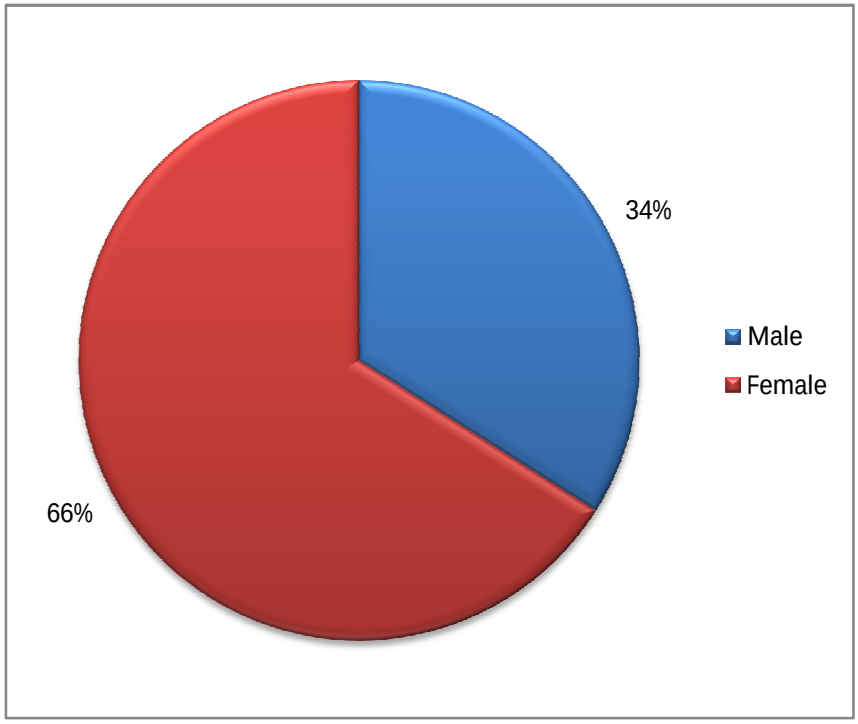


TABLE NO: 3. GENDER DISTRIBUTION OF DACRYOCYSTITIS CASES

Total number of patients	Male		Female	
100	No	Percentage	No	Percentage
	34	34%	66	66%

Gender distribution of dacryocystitis cases.

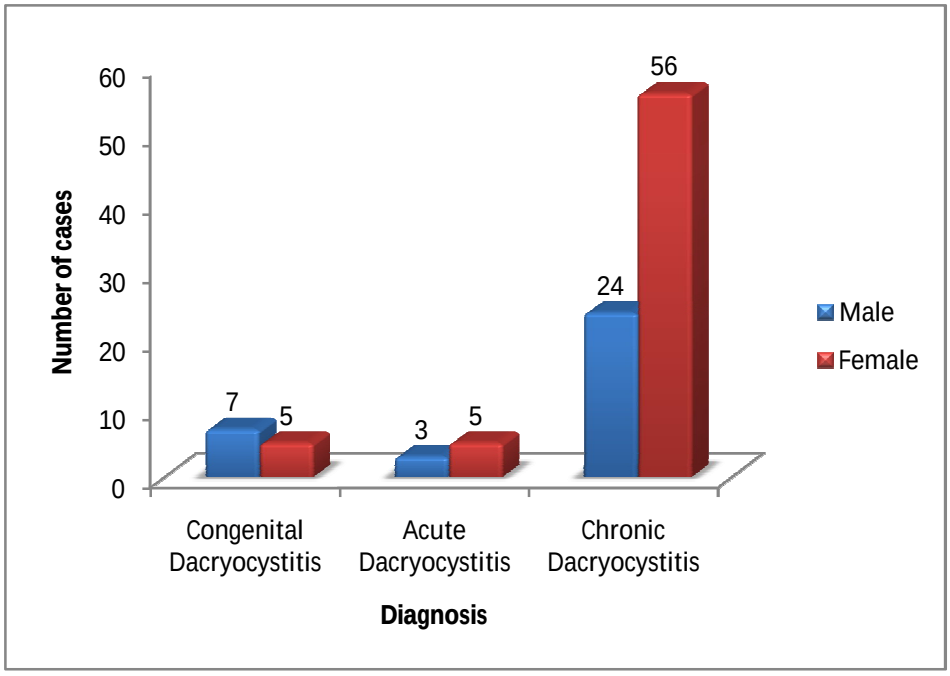


Out of 100 cases under the study, it is observed that females (66%) were more affected than males (34%). The female**:** male ratio is 1.9**:**1

TABLE NO: 4. DISTRIBUTION OF DACRYOCYSTITIS CASES WITH RESPECT TO GENDER AND CLINICAL DIAGNOSIS.

Gender	No of cases			Total	
	Congenital dacryocystitis	Acute dacryocystitis	Chronic dacryocystitis		
Male	7	3	24	34	P value >0.05
Female	5	5	56	66	
Total	12	8	80	100	

Distribution of dacryocystitis cases with respect to gender and clinical diagnosis.

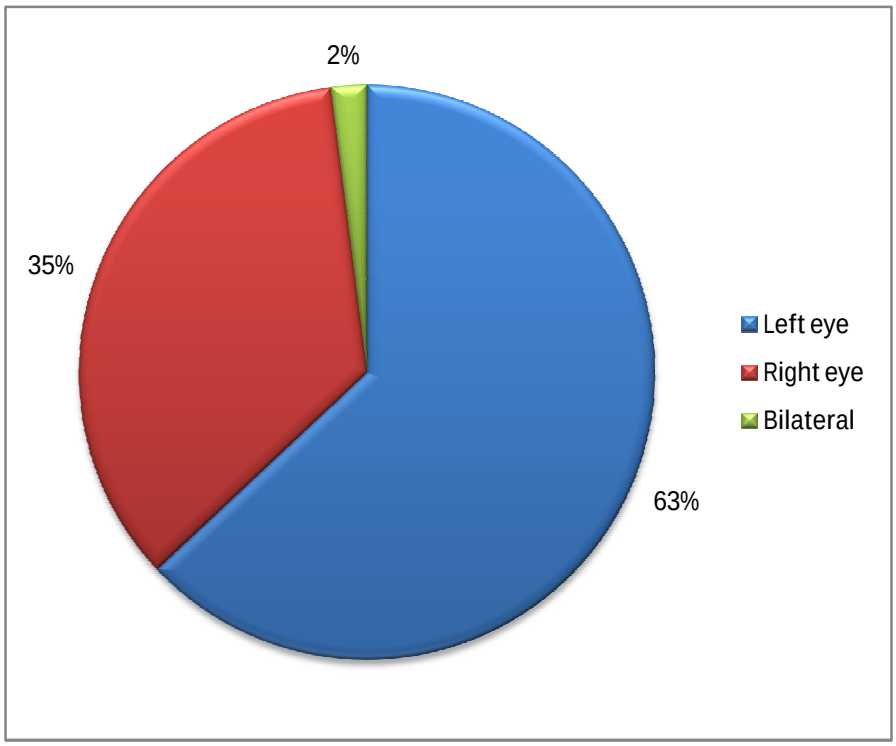


The association between the gender and the clinical diagnosis of dacryocystitis was tested by using Chi square (χ^2) test. The P value is >0.05. This denotes there is no significant correlation between the gender and the clinical type of dacryocystitis.

TABLE NO:5. DISTRIBUTION OF DACRYOCYSTITIS CASES ACCORDING TO THE EYE AFFECTED

Side affected	No of cases	Percentage
Left eye	63	63
Right eye	35	35
Bilateral	2	2
Total	100	100

Distribution of dacryocystitis cases according to the eye affected



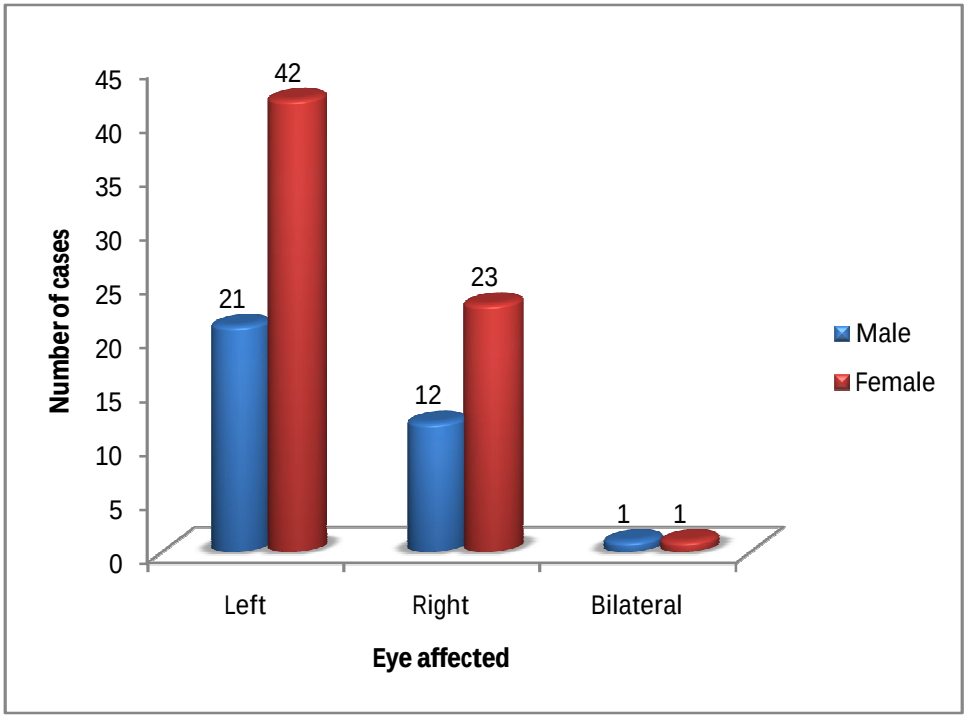
From these findings it is observed that in this study, left eye (63%) is more affected than the right eye (35%).

2% of the cases were bilateral.

TABLENO:6. ANALYSIS OF DISTRIBUTION OF DACRYOCYSTITIS CASES ACCORDING TO GENDER AND EYE AFFECTED

Gender	Eye affected(No of cases)			Total	P value >0.05
	Left	Right	Bilateral		
Male	21	12	1	34	
Female	42	23	1	66	
Total	63	35	2	100	

Distribution of dacryocystitis cases according to gender and eye affected

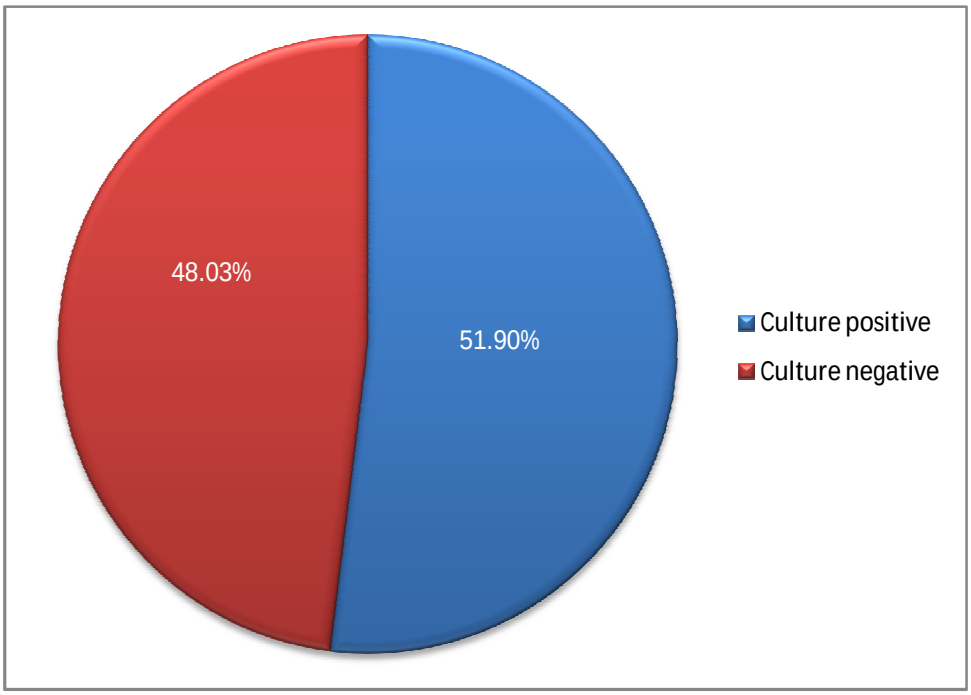


The association between the gender and the eye affected was tested by using Chi square (χ^2) test. The P value is >0.05. This denotes there is no significant correlation between the gender and the eye affected.

TABLE NO: 7. CULTURE POSITIVITY IN DACRYOCYSTITIS CASES

Total no of cases	Total no of samples	Culture positive		Culture negative	
		Number	Percentage	Number	Percentage
100	102	53	51.9%	49	48.03%

Percentage of culture positivity in dacryocystitis cases

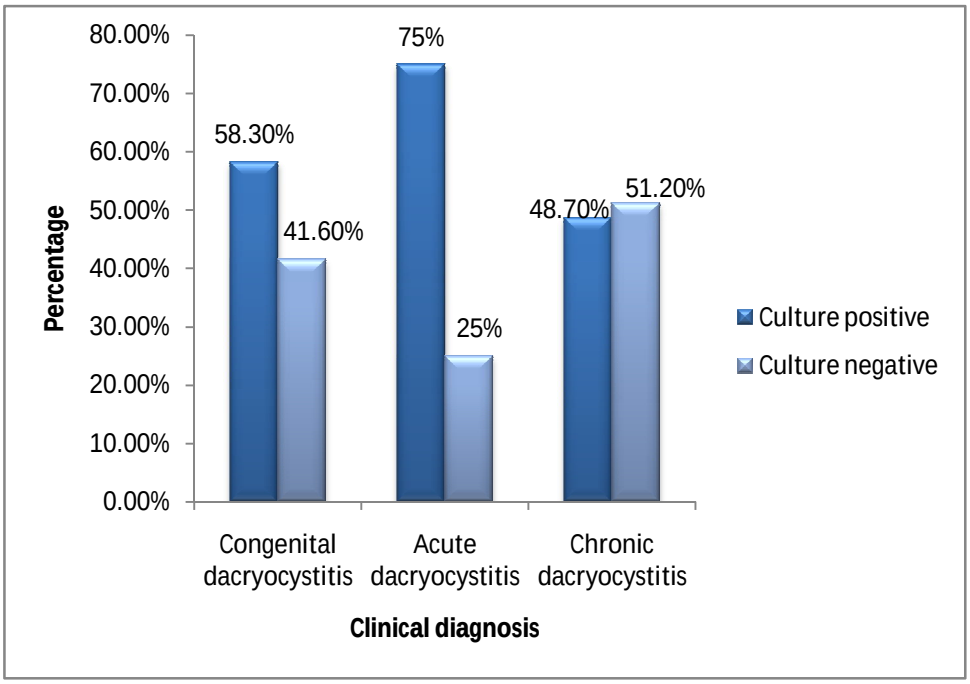


From the above findings it is observed that in this study the overall culture positivity among dacryocystitis cases was 51.9%.

TABLE NO: 8. ANALYSIS OF CULTURE POSITIVITY AMONG CONGENITAL, ACUTE AND CHRONIC DACRYOCYSTITIS CASES

Clinical type	Total no of cases	Total no of samples	Culture positive		Culture negative	
			Number	Percentage	Number	Percentage
Congenital dacryocystitis	12	12	7	58.3%	5	41.6%
Acute dacryocystitis	8	8	6	75%	2	25%
Chronic dacryocystitis	80	82	40	48.7%	42	51.2%

Analysis of culture positivity among congenital, acute and chronic dacryocystitis cases

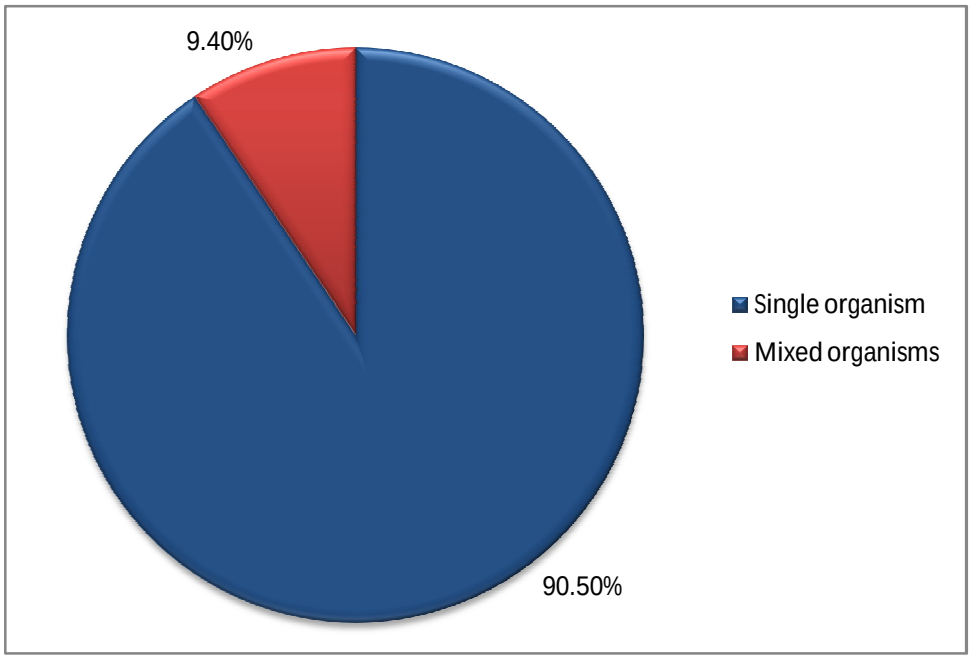


From these findings it is observed that culture positivity was high in acute dacryocystitis (75%) followed by congenital dacryocystitis (58.3%) and chronic dacryocystitis (48.7%).

TABLE NO: 9. DISTRIBUTION OF SINGLE AND MIXED GROWTH ISOLATED FROM DACRYOCYSTITIS CASES

Total no of culture positive samples	Single/Mixed growth	No. of samples	Percentage	P value <0.05
53	Single organism	48	90.5%	
	Mixed organisms	5	9.4%	

Distribution of single and mixed growth isolated from dacryocystitis cases

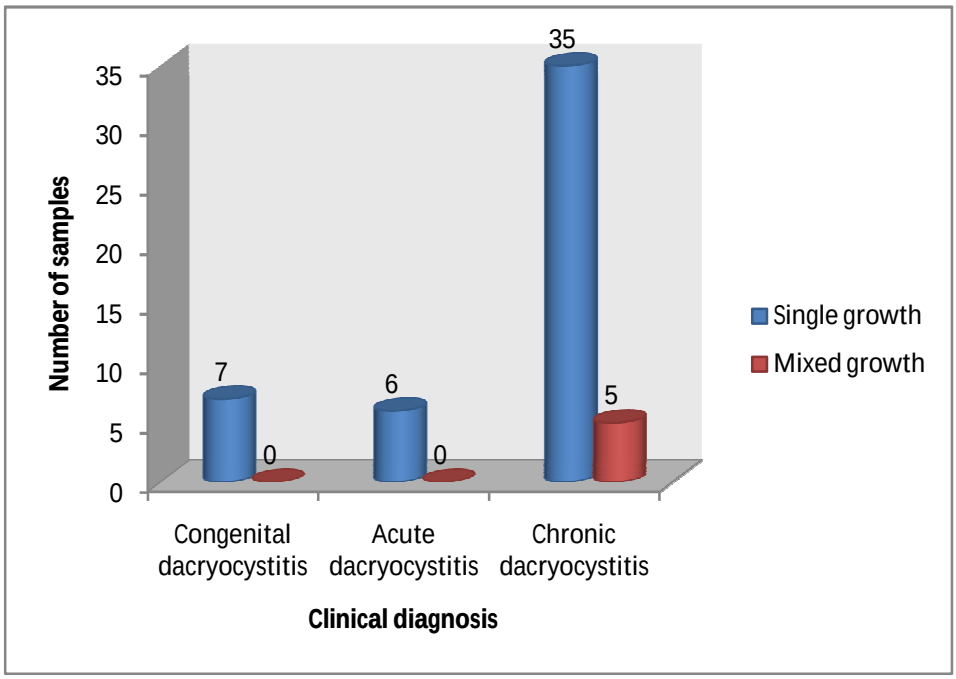


In this study, out of 53 total culture positive samples, 90.5% yielded single organism and 9.4% yielded mixed organisms. It is statistically significant (P value <0.05).

TABLE NO: 10. DISTRIBUTION OF SINGLE AND MIXED GROWTH AMONG CONGENITAL, ACUTE AND CHRONIC DACRYOCYSTITIS CASES

Single/Mixed growth	No of culture positive samples			Total
	Congenital dacryocystitis	Acute dacryocystitis	Chronic dacryocystitis	
Single growth	7	6	35	48
Mixed growth	-	-	5	5
Total	**7**	**6**	**40**	**53**

Distribution of single and mixed growth among congenital, acute and chronic dacryocystitis cases



From the above findings it is observed that in this study mixed growth was present in chronic dacryocystitis cases only.

TABLE NO: 11. DISTRIBUTION OF MIXED ISOLATES

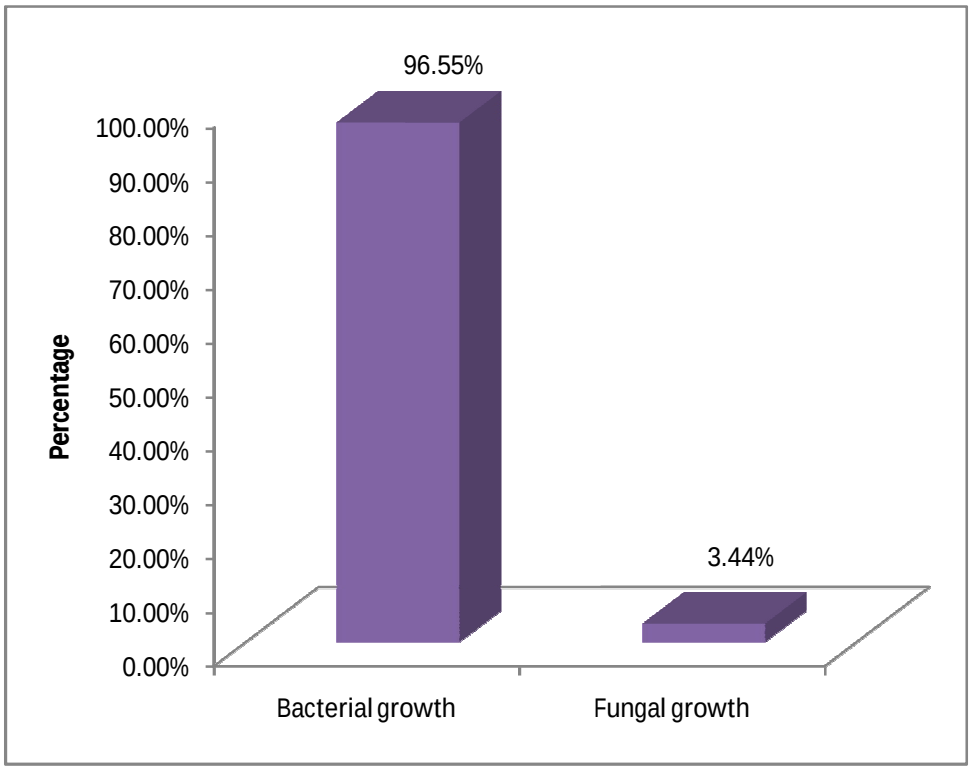
Organisms	No of cases
Staphylococcus aureus + Candida albicans	1
Escherichia coli + Staphylococcus epidermidis	1
Klebsiella pneumoniae + Staphylococcus epidermidis	1
Klebsiella pneumoniae + Staphylococcus aureus	1
Pseudomonas aeruginosa + Staphylococcus epidermidis	1

From the above findings, it is observed that, among 5 mixed isolates, mixed bacterial growth was present in 4 cases and mixed bacterial and fungal growth was present in 1 case.

TABLE NO: 12. DISTRIBUTION OF BACTERIAL AND FUNGAL ISOLATES AMONG DACRYOCYSTITIS CASES.

Type of growth	No of isolates		Total no of isolates	Percentage	
	Single growth	Mixed growth			
Bacterial	47	9	56	96.5%	P value<0.05
Fungal	1	1	2	3.4%	
Total	48	10	58	100%	

Distribution of bacterial and fungal isolates among dacryocystitis cases.

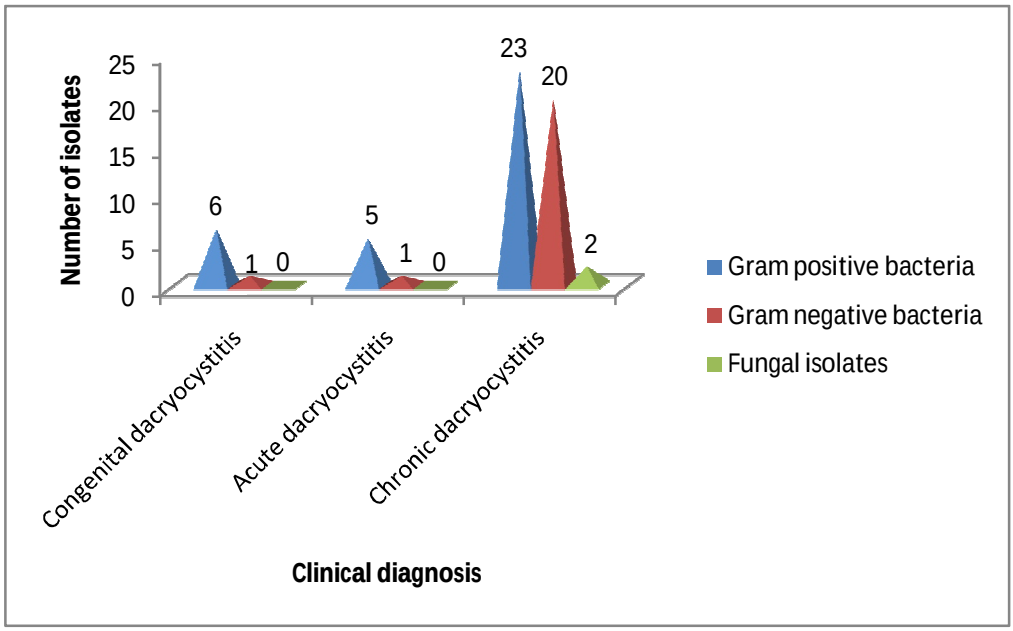


From the above findings it is observed that in this study bacterial growth was predominant and it is statistically significant (P value <0.05).

TAB NO: 13. DISTRIBUTION OF GRAM POSITIVE & GRAM NEGATIVE BACTERIA AND FUNGAL ISOLATES AMONG CONGENITAL, ACUTE AND CHRONIC DACRYOCYSTITIS CASES

Microorganism	Number of isolates			Total	Percentage
	Congenital dacryocystitis	Acute dacryocystitis	Chronic dacryocystitis		
Gram positive bacteria	6	5	23	34	58.6%
Gram negative bacteria	1	1	20	22	37.9%
Fungal isolates	-	-	2	2	3.4%
Total	**7**	**6**	**45**	**58**	**100%**

Distribution of Gram positive &Gram negative bacteria and fungal isolates among congenital, acute and chronic dacryocystitis cases

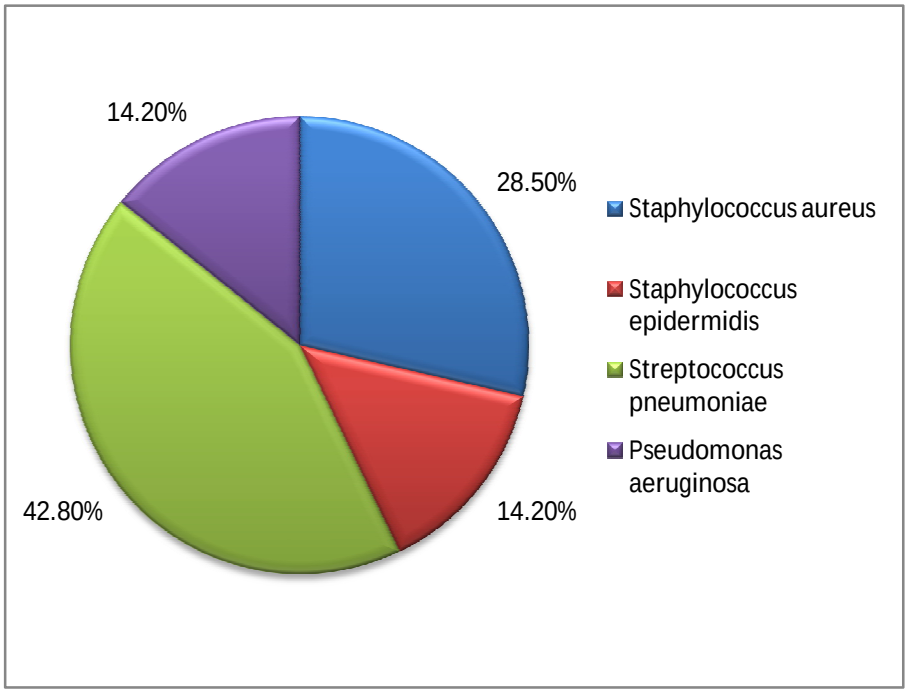


From the above findings it is observed that Gram positive bacteria were the predominant isolates in all the clinical types of dacryocystitis. Fungi were isolated only from chronic cases.

TABLE NO:14. DISTRIBUTION OF BACTERIAL ISOLATES IN CONGENITAL DACRYOCYSTITIS CASES.

Total no of cases/samples	No of organisms isolated	Organism	No of isolates	Percentage
12	7	*Staphylococcus aureus*	2	28.5%
		Staphylococcus epidermidis	1	14.2%
		Streptococcus pneumoniae	3	42.8%
		Pseudomonas aeruginosa	1	14.2%

Distribution of bacterial isolates in congenital dacryocystitis cases.

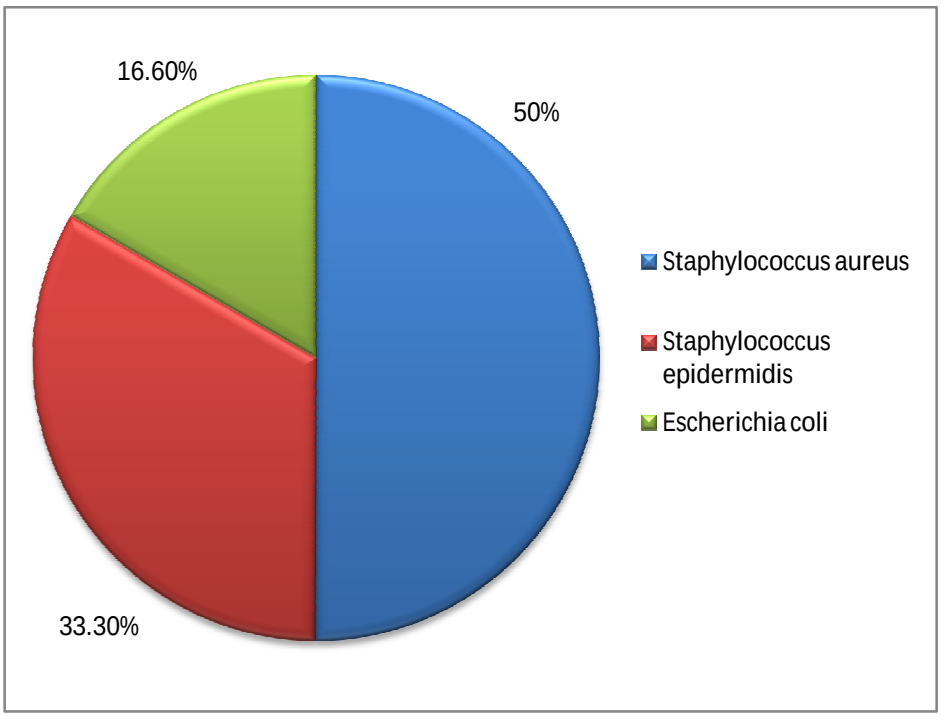


From the above findings it is observed that in this study, the predominant organism isolated from congenital dacryocystitis cases is *Streptococcus pneumoniae*.

TABLE NO: 15. DISTRIBUTION OF BACTERIAL ISOLATES IN ACUTE DACRYOCYSTITIS CASES.

Total no of cases/ samples	No of organisms isolated	Organism	No of isolates	Percentage
8	6	*Staphylococcus aureus*	3	50%
		Staphylococcus epidermidis	2	33.3%
		Escherichia coli	1	16.6%

Distribution of bacterial isolates in acute dacryocystitis cases.

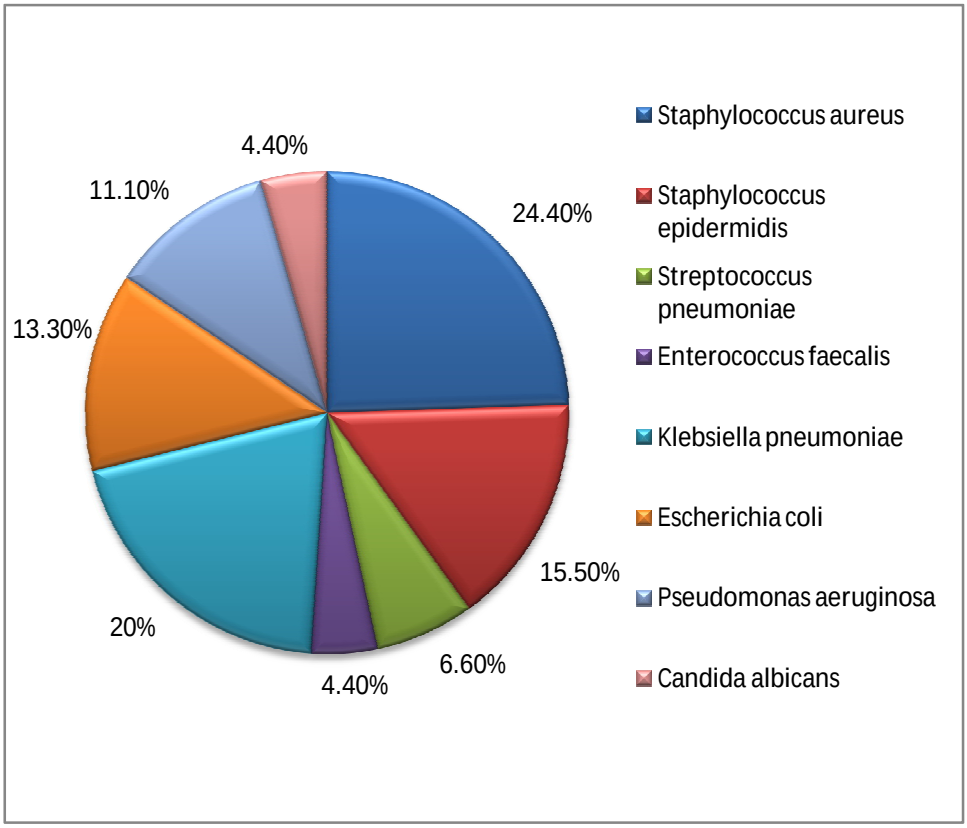


From the above findings it is observed that in this study, the predominant organism isolated from acute dacryocystitis cases is *Staphylococcus aureus*.

TABLE NO: 16. DISTRIBUTION OF BACTERIAL AND FUNGAL ISOLATES IN CHRONIC DACRYOCYSTITIS CASES.

Total no of cases	Total no of samples	No of organisms isolated	Organism	No of isolates	Percentage
80 (78 unilateral & 2 bilateral cases)	82	45	*Staphylococcus aureus*	11	24.4%
			Staphylococcus epidermidis	7	15.5%
			Streptococcus pneumoniae	3	6.6%
			Enterococcus faecalis	2	4.4%
			Klebsiella pneumoniae	9	20%
			Escherichia coli	6	13.3%
			Pseudomonas aeruginosa	5	11.1%
			Candida albicans	2	4.4%

Distribution of bacterial and fungal isolates in chronic dacryocystitis cases.



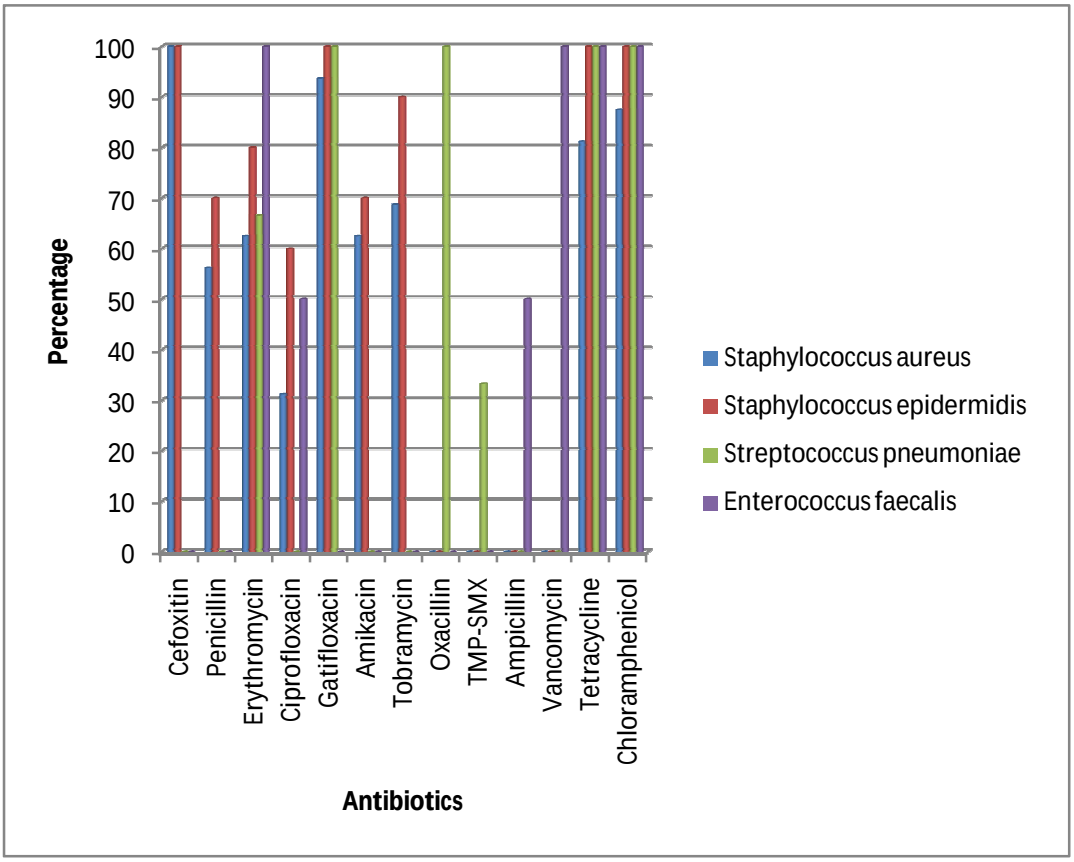
From the above findings it is observed that in this study *Staphylococcus aureus* (24.4%) was the predominant organismwg isolated from chronic dacryocystitis cases. *Klebsiella pneumoniae* (20%) was the common Gram negative bacteria isolated. *Candida albicans* was isolated from 2 cases of chronic dacryocystitis.

TABLE NO: 17.ANTIBIOTIC SENSITIVITY PATTERN OF GRAM POSITIVE BACTERIAL ISOLATES

Antibiotic	***Staphylococcus aureus*** (n = 16)		***Staphylococcus epidermidis***(n = 10)		***Streptococcus pneumoniae*** (n = 6)		***Enterococcus faecalis***(n = 2)	
	S	%	S	%	S	%	S	%
Cefoxitin	16	100	10	100	-	-	-	-
Penicillin	9	56.2	7	70	-	-	-	-
Erythromycin	10	62.5	8	80	4	66.6	2	100
Ciprofloxacin	5	31.2	6	60	-	-	1	50
Gatifloxacin	15	93.7	10	100	6	100	-	-
Amikacin	10	62.5	7	70	-	-	-	-
Tobramycin	11	68.7	9	90	-	-	-	-
Oxacillin	-	-	-	-	6	100	-	-
TMP-SMX	-	-	-	-	2	33.3	-	-
Ampicillin	-	-	-	-	-	-	1	50
Vancomycin	-	-	-	-	-	-	2	100
Tetracycline	13	81.2	10	100	6	100	2	100
Chloramphenicol	14	87.5	10	100	6	100	2	100

TMP-SMX -Trimethoprim- Sulfamethoxazole; S- sensitive

Antibiotic sensitivity pattern of gram positive bacterial isolates



It is observed that in our study, Gatifloxacin was the highly sensitive antibiotic against all Gram positive bacteria tested. Ciprofloxacin and Trimethoprim- Sulfamethoxazole were the least sensitive antibiotics against the Gram positive bacteria tested.

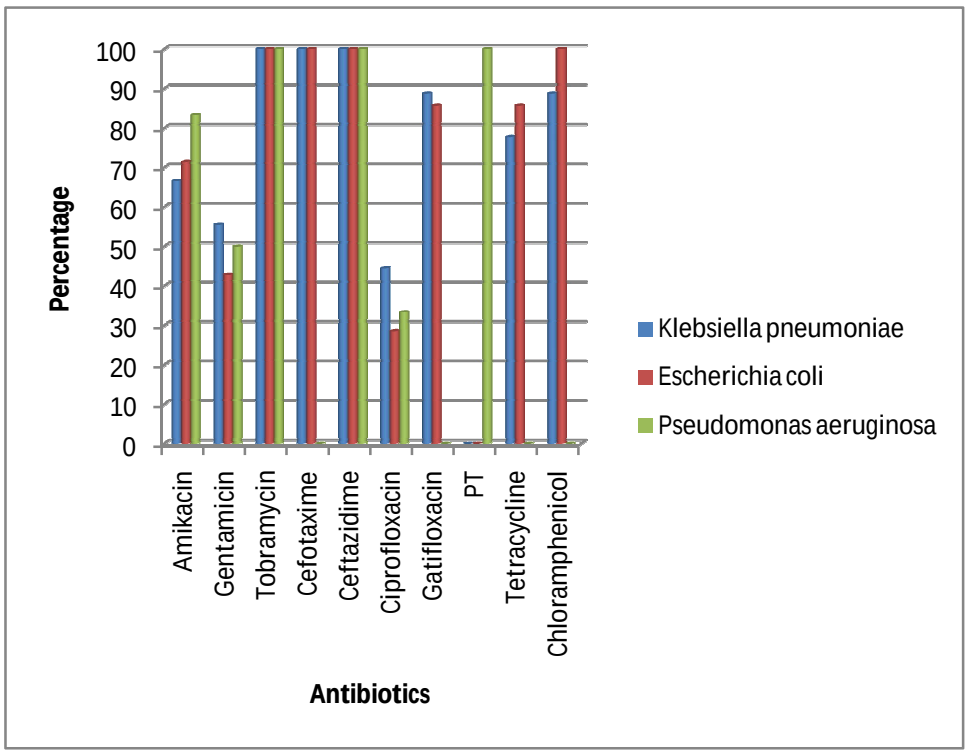
In our study, Methicillin resistance was not detected among *Staphylococci* isolates.

TABLE NO: 18. ANTIBIOTIC SENSITIVITY PATTERN OF GRAM NEGATIVE BACTERIAL ISOLATES

Antibiotic	***Klebsiella pneumoniae*** (n = 9)		***Escherichia coli*** (n = 7)		***Pseudomonas aeruginosa*** (n = 6)	
	S	%	S	%	S	%
Amikacin	6	66.6	5	71.4	5	83.3
Gentamicin	5	55.5	3	42.8	3	50
Tobramycin	9	100	7	100	6	100
Cefotaxime	9	100	7	100	-	-
Ceftazidime	9	100	7	100	6	100
Ciprofloxacin	4	44.4	2	28.5	2	33.3
Gatifloxacin	8	88.8	6	85.7	-	-
PT	-	-	-	-	6	100
Tetracycline	7	77.7	6	85.7	-	-
Chloramphenicol	8	88.8	7	100	-	-

PT-Piperacillin-tazobactam ; S- sensitive

Antibiotic sensitivity pattern of gram negative bacterial isolates



Antibiotic sensitivity pattern showed, all Gram negative bacteria were highly sensitive to Tobramycin. The least sensitive antibiotics against all Gram negative organisms were, Ciprofloxacin and Gentamicin.

In our study, ESBL production was not detected among *Escherichia coli* and *Klebsiella pneumoniae* isolates.

ANTIFUNGAL SUSCEPTIBILITY PATTERN OF FUNGAL ISOLATES

In our study two Candida albicans were isolated from chronic dacryocystitis cases. Both were sensitive to the tested antifungal drugs - Amphotericin B, Fluconazole, Voriconazole and Itraconazole.

DISCUSSION

Dacryocystitis is one of the most common infections of the eye. It can be congenital or acquired. Congenital dacryocystitis affects both sexes equally. Acquired dacryocystitis is common in females[15]. There are multiple factors which play a role in acquiring infection of the lacrimal drainage system. The nasolacrimal system is lined by mucus lined tract contiguous with conjunctiva and nasal mucosa that are normally colonised with bacteria.

Lacrimal apparatus is concerned with secretion and draining of tears, which keep the cornea moist and also protects against airborne pathogens and foreign bodies [34]. Obstruction of the nasolacrimal duct results in stasis of tears, desquamated cells and mucous secretions above the level of obstruction which creates a fertile environment for secondary bacterial infection [5]. Both acute dacryocystitis and chronic dacryocystitis pose a constant threat to cornea and orbital soft tissue. Dacryocystitis is mostly caused by bacteria. Fungal infections caused by *Candida albicans* and *Aspergillus species* occur infrequently[31]. The bacterial agents of dacryocystitis are variable in congenital, acute or chronic cases. However pathogens implicated in dacryocystitis are variable from place to place depending upon the local climate conditions. So it is very important to know the pathogens region wise in management of this condition. Our study was performed to know the bacterial and fungal aetiology of congenital, acute and chronic dacryocystitis and their in vitro antibiotic susceptibility pattern and antifungal susceptibility pattern.

AGE DISTRIBUTION:

Dacryocystitis generally affects two age groups, infants and adult females over 40 years of age [1]. In our study acquired dacryocystitis was common in the age group of 41-50 years. The mean age for dacryocystitis is 47.97 years (Table 1).

This finding is in concordance with study done by Suharshi Gupta et al in which acquired dacryocystitis was common in the age group of 41-50 years[52].

Similarly JyotiBhuyan et al [53] and Shah CP et al [36]in their study found that chronic dacryocystitis was more common in the age group of 41-50 years.

The mean age for acquired dacryocystitis was 49.82 years in the study done by IndrajitSarkar et al[32].

CLINICAL TYPE:

In our study chronic dacryocystitis (80%) was more common than acute one (8%). Congenital dacryocystitis accounts for 12% of cases (Table 2).

This finding correlates with study done by Bharathi et al [38] in which 70.1% had chronic onset and 29.9% patients had acute onset dacryocystitis. Similarly Syed Ali RazaRizvi et al[31] reported 81.3% of chronic dacryocystitis and 18.7% of acute dacryocystitis.

Prakash et al[35] studied congenital and acquired dacryocystitis cases and reported 63.75% cases of chronic dacryocystitis, 25% cases of acute dacryocystitis and 11.25% cases of congenital dacryocystitis.

Acute dacryocystitis invariably led to chronic dacryocystitis, so chronic form is more common.

GENDER DISTRIBUTION:

In our study females (66%) were more affected than males (34%), the ratio being 1.9**:**1 (female**:** male). (Table 3&4)

This finding correlates with study done by Delia A Ch et al [54] in which among 421 cases of chronic dacryocystitis, 61.04% were females and 38.95% were males. Chaudhry IA et al [55] reported in their study that females (65.4%) were more affected than males (34.6%).

Similarly Sarkar I et al[32] in their study reported a female to male ratio of 2.1**:**1 and Badhu B et al[56]reported female to male ratio of 2**:**1.

The female predilection may be due to presence of narrow nasolacrimal duct in females, usage of kajal by females and hormonal factors [35, 57]**.**

LATERALITY:

In our study left eye (63%) was more commonly affected than the right eye (35%) and 2% of cases were bilateral (Table 5&6).

This finding correlates with the study done by Khevna Patel et al[2] in which dacryocystitis was more common in the left eye (56%) than the right eye(44%). Prakash R et al[35] found in their study that there was higher incidence of dacryocystitis on left side (50%) as compared to right side(40%) and 10% of the cases were bilateral.

The predilection of the left side is possibly because the nasolacrimal duct and the lacrimal fossa form a wider angle on the right side than on the left side [14].

CULTURE POSITIVITY:

In our study, the overall culture positivity was 51.9 % (Table7). Culture positivity in acute, chronic and congenital dacryocystitis cases were 75%, 48.7% and 58.3% respectively (Table 8).

This finding correlates with study done by Syed Ali RazaRizvi et al[31] in which the percentage of culture positivity was 76.47% in acute cases and 37.84% in chronic cases.

Madhusudhan et al [58] studied 23 patients of acute dacryocystitis and culture positivity was 69.5%. Shwetha B A et al[3] studied 200 patients of chronic dacryocystitis. The percentage of culture positivity was 57.5%.

Andreas Kuchar et al**[41]** studied 47 cases of congenital dacryocystitis and culture positivity was 72.64%.

NUMBER OF ORGANISMS:

In our study, single organism was isolated from 90.5% of culture positive cases. Mixed organisms were isolated from 9.4% of culture positive cases (Table 9). All the mixed isolates were from chronic dacryocystitis cases(Table 10).The mixed growth was predominantly bacterial isolates (Table11).

This finding correlates with the study done by Shah C P et al [36] in which single organism was isolated from 90.9% and mixed growth was isolated from 9.1% of culture positive cases. Similarly, Bharathi et al [38] reported 93.8% of single organism and 6.2% of mixed isolates.

A higher incidence of mixed isolates was reported by Chaudhary M et al [37] (14.1%), JithendraKandati et al[5] (14.5%).

BACTERIAL AND FUNGAL PROFILE OF DACRYOCYSTITIS

The spectrum of microorganisms causing dacryocystitis may vary from region to region.

In our study, bacterial growth (96.5%) was more common than fungal growth (3.4%) (Table 12).

Similarly, Jithendra Kandati et al [5] and Bahram Eshraghi et al **[33]** reported in their study that bacterial growth was common than fungal growth in dacryocystitis cases.

In our study, Gram positive bacteria predominate in congenital, acute and chronic dacryocystitis cases. Overall Gram positive bacteria, Gram negative bacteria and fungal isolates account for 58.6%, 37.9% and 3.4% of culture positives respectively. The fungal growth was present only in chronic cases (Table 13).

This finding coincides with the following studies. Kebede et al [59] reported 62.6% of Gram positive bacteria and 37.4% of Gram negative bacteria.

Similarly Prakash et al[35] reported 64.8% of Gram positive bacteria and 35.11% of Gram negative bacteria.

Mills et al[60] reported 68.8% of Gram positive bacteria and 28.7% of Gram negative bacteria. A higher incidence of Gram positive bacteria of 69% and 78.57% was reported by Hartikainen J et al[61] and IndrajitSarkar et al[32]respectively.

Congenital dacryocystitis:

In our study, *Streptococcus pneumoniae* (42.8%) was the common organism isolated from congenital dacryocystitis cases, followed by *Staphylococcus aureus*(28.5%), *Staphylococcus epidermidis*(14.2%) and *Pseudomonas aeruginosa*(14.2%)(Table 14).

This finding correlates with the study done by, Andreas Kuchar et al[41] who studied 47 cases of congenital dacryocystitis and the most common organism isolated was *Streptococcus pneumoniae*(35.4%).

Similarly, UmeshBareja et al[62] and Usha K et al[63]reported *Streptococcus pneumoniae* as the most common organism isolated from congenital dacryocystitis cases.

Acute dacryocystitis:

In our study, *Staphylococcus aureus*(50%) was the most common organism isolated from acute cases, followed by *Staphylococcus epidermidis*(33.3%) and *Escherichia coli*(16.6%)(Table 15).

This finding correlates with the study done by BahramEshraghi et al[33] in which *Staphylococcus aureus* was the predominant organism isolated from acute dacryocystitis cases (35%).

Similarly, Bharathi et al[38] and Ali MJ et al [64] reported in their study that *Staphylococcus aureus* was the predominant organism in acute dacryocystitis cases.

Chronic dacryocystitis:

In our study, *Staphylococcus aureus*(24.4%) was the predominant organism isolated from chronic cases, followed by *Klebsiella pneumoniae* (*20%*), *Staphylococcus epidermidis*(15.5%), *Escherichia coli* (13.3%), *Pseudomonas aeruginosa*(11.1%), *Streptococcus pneumoniae* (6.6%), *Enterococcus faecalis*(4.4%)and *Candida albicans*(4.4%) (Table 16).

This finding correlates with the study done by Shah C P et al **[36]** in which *Staphylococcus aureus*(27%) was the predominant organism isolated from chronic cases. Similarly, Shwetha B A et al[3] reported *Staphylococcus aureus*(32.1%) as the predominant organism isolated from chronic cases.

Sun X et al[65] and Mandal et al[39]also reported *Staphylococcus aureus* as the common organism in chronic dacryocystitis cases accounting for 34.5% and 40% respectively.

However in studies done by BahramEshraghi et al[33] and Razavi et al[66], *Staphylococcus epidermidis* was the predominant organism in chronic dacryocystitis cases.

In our study *Enterococcus faecalis*(4.4%) was isolated in chronic cases. Similarly, Briscoe D et al [67] and Indrajit Sarkar et al [32] reported 7% and 1.1% of *Enterococcus* species respectively.

Klebsiella pneumoniae was the predominant Gram negative bacteria isolated in our study. Similarly Shwetha B A et al[3], Bahram Eshraghi et al**[33]**reported *Klebsiella pneumoniae* as common Gram negative bacteria in chronic cases.

However Assefa Y et al[68], Shah CP et al[36] , Mandal et al[39] and JyotiBhuyan et al[53] reported *Pseudomonas aeruginosa* as common Gram negative bacteria in chronic cases.

In our study, *Candida albicans* was isolated from two cases of chronic dacryocystitis. Similarly Brook et al [69] reported 5% of fungal isolates in dacryocystitis cases, of which 3.3% was *Candida albicans*. Jithendra Kandati et al [5] reported 0.6% of *Candida albicans* out of 314 isolates.

Obi et al [70] reported a case of dacryocystitis caused by Candida dubliniensis in a neutropenic patient.

BahramEshraghi et al **[33]**in their study reported 2% of *Aspergillus species* in dacryocystitis cases.

ANTIBIOTIC SENSITIVITY PATTERN

Gram positive bacteria (Table 17):

In our study, Gatifloxacin was the highly sensitive antibiotic against all Gram positive bacteria tested. Ciprofloxacin and Trimethoprim-Sulfamethoxazole were the least sensitive antibiotics against Gram positive bacteria tested.

Similarly IndrajitSarkar et al[32] and Bharathi et al[38] reported Gatifloxacin as the highly sensitive antibiotic against Gram positive bacteria.

Assefa Y et al [68] and Bharathi et al [38] in their study reported ciprofloxacin as least sensitive antibiotic against Gram positive bacteria.

In our study Methicillin resistance was not detected among *Staphylococci* isolates.

Gram negative bacteria (Table 18):

In our study, all Gram negative bacteria were highly sensitive to Tobramycin. All *Klebsiella pneumoniae* and *Escherichia coli* isolates were 100% sensitive to Cefotaxime and Ceftazidime. All *Pseudomonas aeruginosa* isolates were 100% sensitive to Ceftazidime and Piperacillin-tazobactam. The least sensitive antibiotics against all Gram negative bacteria were, Ciprofloxacin and Gentamicin.

Similarly Suharshi Gupta et al [52] and JithendraKandati et al [5] reported Tobramycin as most effective antibiotic against Gram negative bacteria.

Bharathi et al[38] and Prakash R et al[35] reported Ciprofloxacin as least effective antibiotic against Gram negative bacteria.

The reason for resistance to Ciprofloxacin and Gentamicin might be due to routine use of these antibiotics for all ocular infections in our population.

In our study, ESBL production was not detected among *Escherichia coli* and *Klebsiella pneumoniae* isolates.

ANTIFUNGAL SUSCEPTIBILITY PATTERN:

In our study both the *Candida albicans* isolates were sensitive to the tested antifungal drugs -Amphotericin B, Fluconazole, Voriconazole and Itraconazole.

SUMMARY

- The present study was carried out at the Institute of Microbiology, Madras Medical College, Chennai, in association with Regional Institute of Ophthalmology and Government Ophthalmic Hospital, Chennai.
- 100 clinically diagnosed patients of dacryocystitis of all age groups were studied from October 2014 to August 2015.
- 12 patients of congenital dacryocystitis, 8 patients of acute dacryocystitis and 80 patients of chronic dacryocystitis were studied.
- Dacryocystitis was more common in the age group of 40 to 50 years.
- Females (66%) were more affected than males (34%).
- Out of 100 patients, 98 patients had unilateral dacryocystitis and 2 patients had bilateral dacryocystitis. Totally 102 samples were collected from 100 patients.
- Left eye (63%) was commonly affected than the right eye (35%).
- 51.9% of samples were culture positive, 48.03% of samples were culture negative.
- The highest culture positivity was found in acute dacryocystitis (75%) followed by congenital dacryocystitis (58.3%) and chronic dacryocystitis (48.7%).
- Among the culture positive samples, 90.5% yielded single organism and 9.4% yielded mixed organisms.

- Mixed organisms were isolated only from chronic dacryocystitis cases.
- Totally, 58 organisms were isolated from 102 samples.
- Among the 58 organisms isolated, 58.6% were Gram positive bacteria, 37.9% were Gram negative bacteria, and 3.4% were fungal isolates.
- Fungal organisms were isolated only from chronic dacryocystitis cases.
- *Streptococcus pneumoniae* (42.8%) was the common organism isolated from congenital dacryocystitis cases, followed by *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa*.
- *Staphylococcus aureus* (50%) was the most common organism isolated from acute dacryocystitis cases, followed by *Staphylococcus epidermidis* and *Escherichia coli.*
- *Staphylococcus aureus*(24.4%) was the common organism isolated from chronic dacryocystitis cases, followed by *Klebsiella pneumoniae, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pneumoniae*, *Enterococcus faecalis* and *Candida albicans.*
- All the *Staphylococci* isolates were Methicillin sensitive.
- All Gram positive bacteria were highly sensitive to Gatifloxacin and least sensitive to Ciprofloxacin.
- ESBL production was not detected among *Escherichia coli* and *Klebsiella pneumoniae* isolates.

- All Gram negative bacteria were highly sensitive to Tobramycin and least sensitive to Ciprofloxacin and Gentamicin.
- The two Candida albicans isolates were sensitive to Amphotericin B, Fluconazole, Voriconazole and Itraconazole.

CONCLUSION

This cross sectional study conducted at the Institute of Microbiology, Rajiv Gandhi Government General Hospital aimed at isolating the bacteria and fungi associated with congenital, acute and chronic dacryocystitis and had revealed that Gram positive bacteria were predominant in all the three types of dacryocystitis cases. *Streptococcus pneumoniae* was the common pathogen in congenital dacryocystitis and *Staphylococcus aureus* was the common pathogen in acute and chronic dacryocystitis. The incidence of Gram negative pathogens were more in chronic dacryocystitis. *Klebsiella pneumoniae* was the common Gram negative pathogen isolated from chronic cases. *Candida albicans* was isolated from two cases of chronic dacryocystitis.

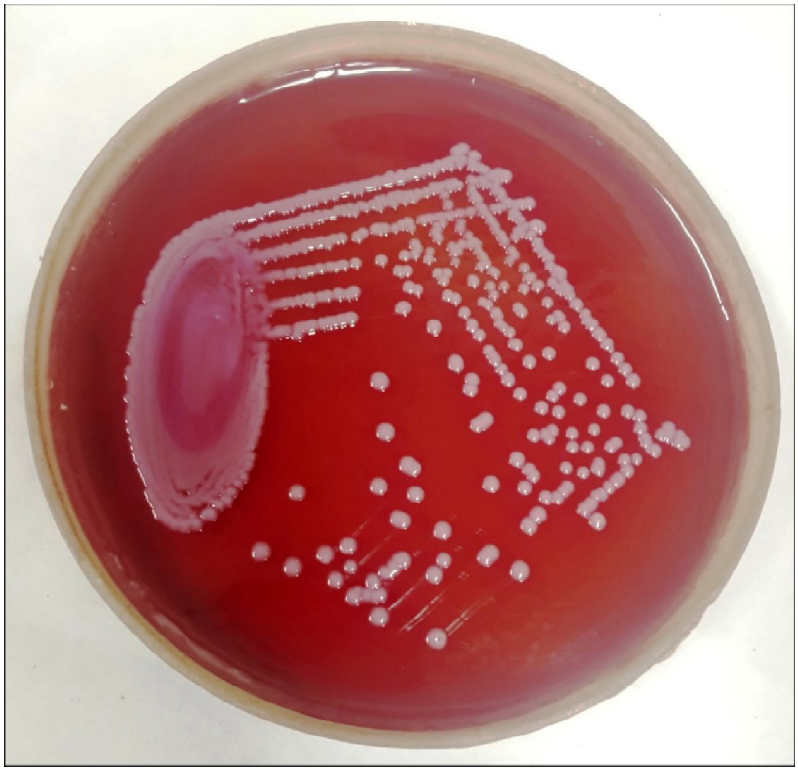
Dacryocystitis pose a constant threat to cornea and orbital soft tissue. There is a change in etiological agents causing dacryocystitis over the time.

Microbial culture and sensitivity when performed in samples from all the patients having dacryocystitis is useful. This would contribute to the choice of appropriate and effective antimicrobial agents.

COLOUR PLATE: 1. CHRONIC DACRYOCYSTITIS WITH MUCOCELE

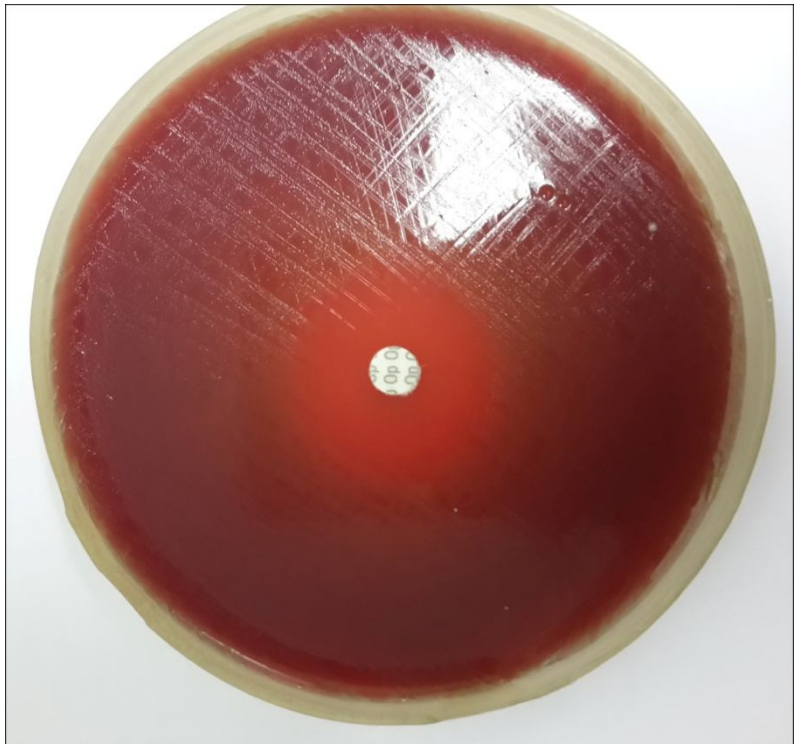
COLOUR PLATE: 2. COLONIES OF *STAPHYLOCOCCUS AUREUS* ON BLOOD AGAR PLATE

COLOUR PLATE: 3. COLONIES OF *KLEBSIELLA PNEUMONIAE* ON MACCONKEY AGAR PLATE

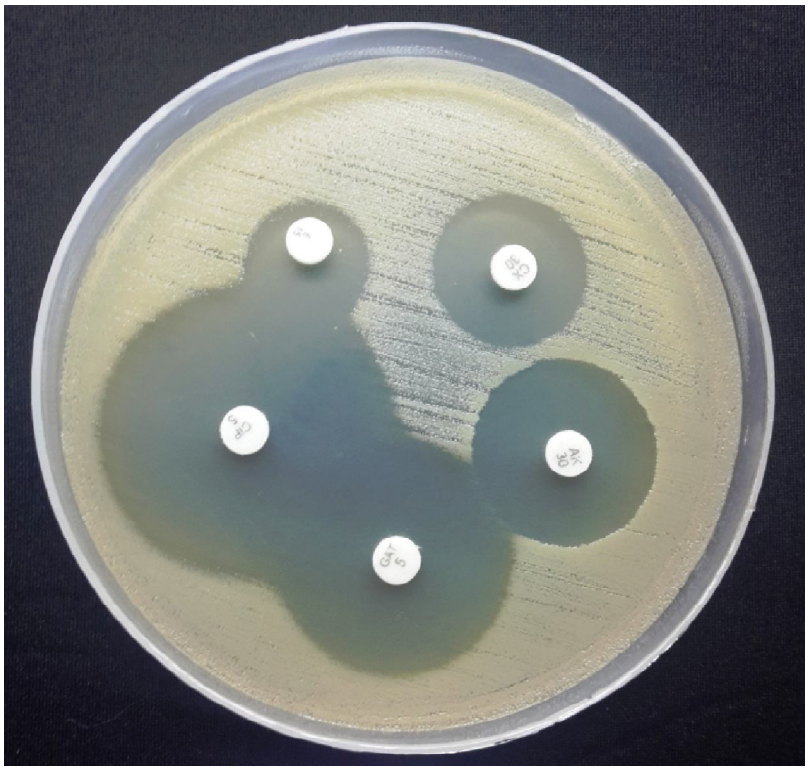


COLOUR PLATE: 4. COLONIES OF *PSEUDOMONAS AERUGINOSA* ON NUTRIENT AGAR PLATE

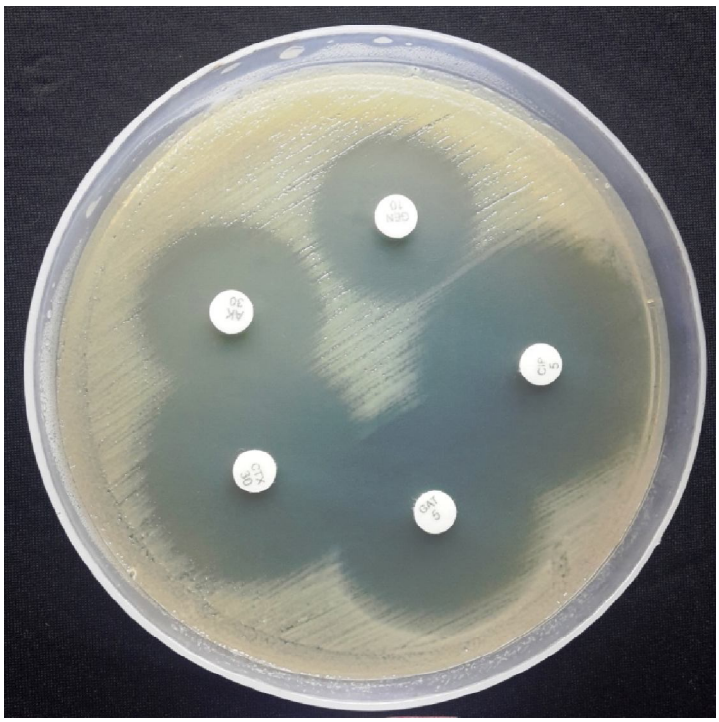
COLOUR PLATE: 5 COLONIES OF *STREPTOCOCCUS PNEUMONIAE* SHOWING OPTOCHIN SENSITIVITY



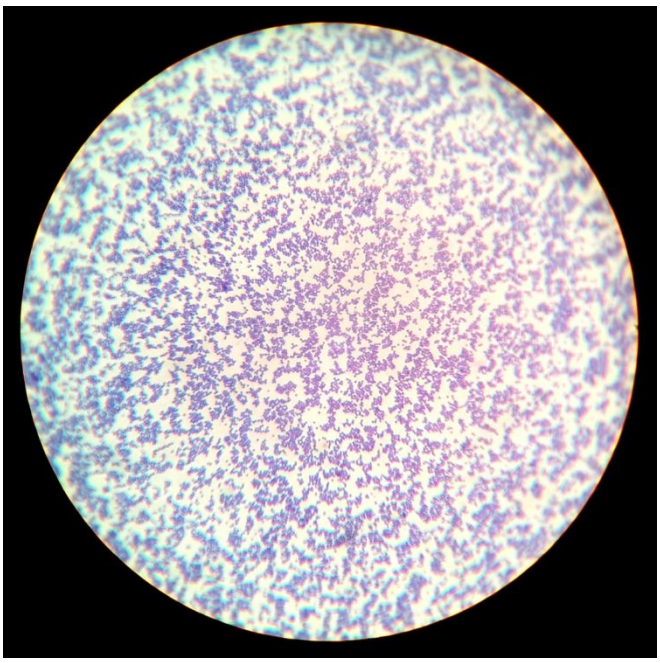
COLOUR PLATE: 6 ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *STAPHYLOCOCCUS AUREUS*



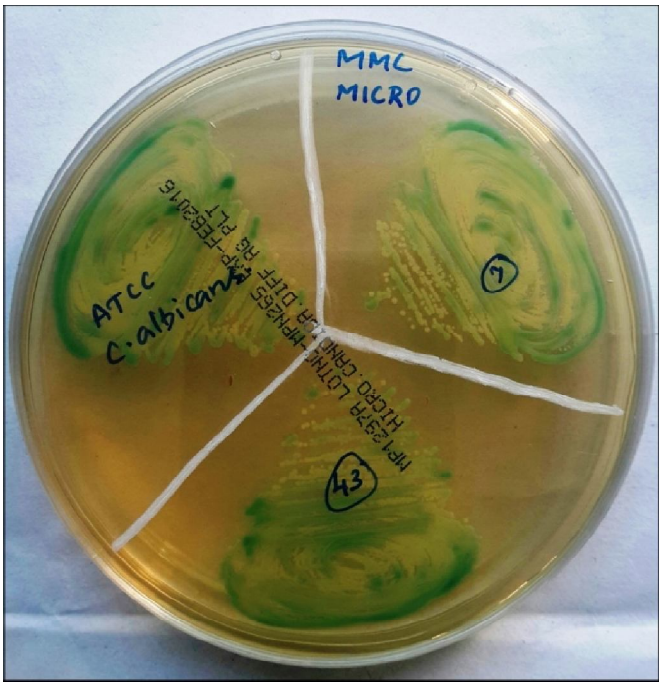
COLOUR PLATE: 7. ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *KLEBSIELLA PNEUMONIAE*



COLOUR PLATE: 8. CULTURE SMEAR OF *STAPHYLOCOCCUS AUREUS*

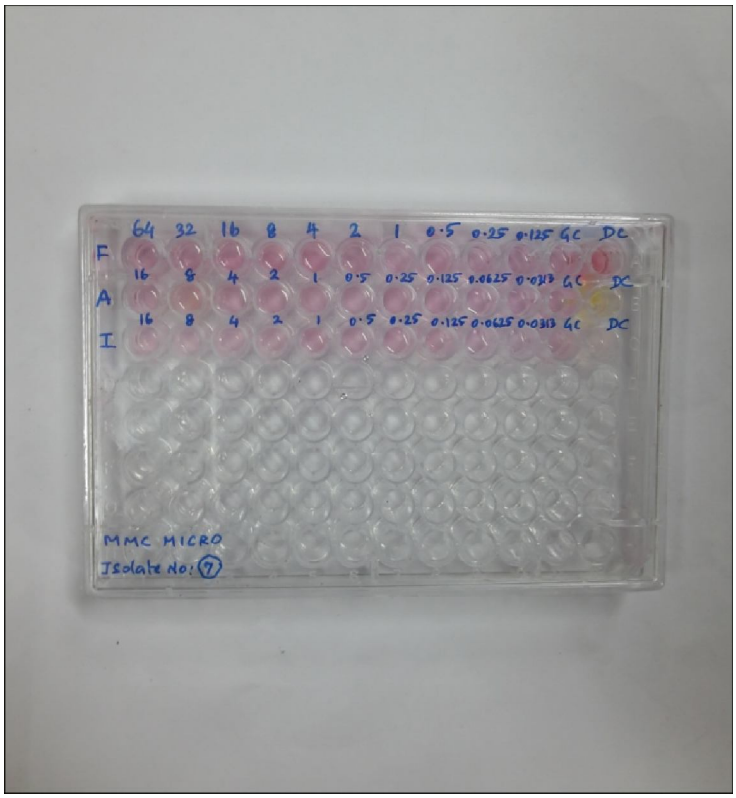


COLOUR PLATE: 9. COLONIES OF CANDIDA ALBICANS ON CHROM AGAR PLATE



COLOUR PLATE: 10. ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ALBICANS BY DISC DIFFUSION METHOD

COLOUR PLATE: 11.ANTIFUNGAL SUSCEPTIBILITY PATTERN OF CANDIDA ALBICANS BY BROTH MICRO DILUTION METHOD



APPENDIX – I

ABBREVIATIONS

ATCC	-	American type culture collection
CLSI	-	Clinical and Laboratory Standards Institute
DCR	-	Dacryocystorhinostomy
DCT	-	Dacryocystectomy
ESBL	-	Extended spectrum beta lactamases
KOH	-	Potassium hydroxide
MR	-	Methyl red
MIC	-	Minimal inhibitory concentration
NLD	-	Nasolacrimal duct
RPMI	-	Rosewell Park Memorial Institute
SDA	-	Sabourauds Dextrose Agar
TSI	-	Triple Sugar Iron
VP	-	Voges -Proskauer

APPENDIX II

A.STAINS AND REAGENTS

I. Gram staining

Methyl violet (2%)	l0g Methyl violet in l00ml absolute alcohol in 1litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbolfuchsin 1%	Secondary stain

II. 10% KOH

Potassium hydroxide	l0g
Glycerol	l0ml
Distilled water	80ml

B.MEDIA USED:

1.MacConkey agar

Peptone	20g
Sodium taurocholate	5g
Distilled Water	1 ltr
Agar	20g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00mI

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix. Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15minutes.

2. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

3. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution**.**

4.Sabouraud dextrose agar

Dextrose	40g
Peptone	l0g
Agar	20g
Distilled water	l000ml

pH = 5.6

Sterilise by autoclaving at 121ºC for 20 min

5. Mueller Hinton Agar:

Beef, infusion	300ml
Casein acid hydrolysate	17.5g
Starch	1.5g
Agar	10g
Distilled water	1 litre

Final pH (at 25°C) 7.3±0.1

Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

6.Corn meal agar

Ingredients

Corn meal	8gm
Agar	4gm
Distilled water	200ml
Tween 80(1%)	2ml

Heat corn meal and water at 60^0C for 1 hour and filter through filter paper. Add distilled water and agar. Autoclave it at 121^0C for 15 minutes and pour in lates. Cornmeal tween agar is prepared by adding Tween 80 before autoclaving.

7.RPMI 1640 Medium

RPMI 1640 medium buffered with 0.165 mol/L MOPS, 1 L10.4 g powdered RPMI 1640 medium (with glutamine and phenol red, without

bicarbonate) 34.53 g MOPS (3-[N-morpholino] propanesulfonic acid) buffer. Dissolve powdered medium in 900 mL distilled H2O. Add MOPS (final concentration of 0.165 mol/L) and stir until dissolved. While stirring, adjust the pH to 7.0 at 25^0C using 1 mol/L sodium hydroxide. Add additional water to bring medium to a final volume of 1 L. Filter sterilize and store at 4 °C until use.

C.MEDIA AND REAGENTS REQUIRED FOR BIOCHEMICAL IDENTIFICATION:

1.Oxidase Reagent

Tetra methyl p-phenylenediaminedihyrochloride- 1% aqueous solution.

2.Catalase test

3% hydrogen peroxide

3.Indole test

Kovac's reagent

Amyl or isoamyl alcohol	150ml
Para dimethyl amino benzaldehyde	10g
Concentrated hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	1g
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml

Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	l0ml
Sterile 20% urea solution	l00ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20g
Bromothymol blue	0.2% 40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6.Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	1g
Lactose	l0g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
Sodiumthiosulphate	0.3g
Agar	12g

Phenol red 0.2% solution	l2ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube.Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7.Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube

Methyl Red Reagent

Methyl Red	10mg
Ethyl alcohol	30ml
Distilled water	20ml

VogesProskauer Reagent

Reagent A:

Alpha naphthol	5g
Ethyl alcohol	100ml

Reagent B:

Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium.

To the basal medium of peptone water, add sterilised sugars, 1%indicator bromothymol blue with Durham's tube.

Basal medium peptone water

Sugar solutions:

Sugar	1ml
Dislilled water	l00ml

pH = 7.6.

9. Potassium nitrate broth

Potassium nitrate (KN03)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclave.

10.Decarboxylase media:

10a.Moeller decarboxylase broth base:

Ingredients	**gms/L**
Peptone	5
Beef extract	5
Bromocresol purple	0.01
Cresol red	0.005
Glucose	0.5
Pyridoxal	0.005

Final pH 6

10b. Aminoacid:

Add 10 g of the levo form of the amino acid for 1000ml.mix and dispense in sterile tubes.

11. Hugh&Leifson's Oxidation –Fermentation test:

Peptone	2g
Sodium chloride	5g
D-glucose	10g
Bromothymol blue	0.03g
Agar	3.0g
Dipotassium phosphate	0.30g
Distilled water	1L

pH =7.1

Basal medium is autoclaved.1% of sterile sugar solutions is added to the basal medium. Dispense into sterile test tubes without slant.

12. Phenolphthalein diphosphate agar

Sterilize 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4ºC.Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50ºCand pour plates. Grow the staphylococcus overnight at 37ºC on the medium. Invert the plate and pour a few drops of ammonia solution SG 0.88 in to the lid. Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

ANNEXURE - I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. S.Shanmuga Sundari,
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr.S.Shanmuga Sundari,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on bacterial and fungal infections in patients with dacryocystitis in a tertiary care hospital". No.18102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

16/10/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE - II

PROFORMA

Name	:	IP /OP no	:
Age	:	Sex	:
Address	:	Occupation	:

Present Complaints and duration :

Past history :

Personal history :

Treatment history :

Clinical diagnosis :

Microbiological investigation

Specimen collected :

Direct examination :

Direct Gram stain :

KOH mount :

Bacterial Culture

MacConkey agar :

Blood agar :

Chocolate agar :

Culture Gram stain :

Motility :

Catalase test :

Coagulase test :

Oxidase test :

Biochemical reactions :

Isolate identified in sample :

Antibiotic susceptibility pattern :

Fungal culture :

Sabouraud’s dextrose agar :

Lactophenol Cotton Blue mount :

Gram stain :

Isolate identified in sample :

Antifungal susceptibility pattern :

ANNEXURE - III

PATIENT CONSENT FORM

Title of the study**:"A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH DACRYOCYSTITIS IN A TERTIARY CARE HOSPITAL".**

Name	:	Date	:
Age	:	OP/IP No	:
Sex	:	Project Patient No	:

Documentation of the informed consent

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I hereby give my consent to be included as a participant in "**A STUDY ON BACTERIAL AND FUNGAL INFECTIONS IN PATIENTS WITH DACRYOCYSTITIS IN A TERTIARY CARE HOSPITAL".**

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment.
6. I have been advised about the risks associated with my participation in this study.
7. I agree to cooperate with the investigator and I will inform him/her immediately if I suffer unusual symptoms.
8. I have not participated in any research study within the past _____ month(s).

9. I am aware of the fact that I can opt out of the study at any time without having to give my reason and this will not affect my future treatment in this hospital.
10. I am also aware that the investigator may terminate my participation in the study at any time, for any reason, without any consent.
11. I hereby give permission to the investigators to release the information obtained from me as result of participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC. I understand that they are publicly presented.
12. I have understood that my identity will be kept confidential if my data are publicly presented.
13. I have had my questions answered to my satisfaction.
14. I have decided to be in the research study. I also give my consent to give my clinical specimen(purulent discharge from lacrimal punctum) for further investigations.

I am aware that if I have any question during this study, I should contact the investigator. By signing this consent form I attest that the information given in this document has been clearly explained to me and understood by me, I will be given a copy of this consent document.

Name and signature / thumb impression of the participant (For age <17 years- Name& signature of the parent/guardian)

Name _____
Signature_____

Date_____

Name and Signature of the investigator

Name _____
Signature_____

Date_____

ANNEXURE – IV

MASTER CHART

SNO	AGE	SEX	OP/IP/MD	UNILATERAL / BILATERAL	SIDE AFFECTED	DIAGNOSIS	GROWTH/NO GROWTH	SINGLE / MIXED GROWTH	ORG.1	ORG.2	ORGANISM -1																						ORGANISM -2																					
											AK	Gs	Cx	Ery	Clp	Gati	TMP-SMX	Gen	Tob	CTX	CAZ	Pen	Tetra	Amp	Chlor	PT	Van	Flu	Rif	Ampho	Vori	AK	Gs	Cx	Ery	Clp	Gati	TMP-SMX	Gen	Tob	CTX	CAZ	Pen	Tetra	Amp	Chlor	PT	Van	Flu	Rif	Ampho	Vori
1	46	F	83296/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																									
2	2	M	505383/IP	Unilateral	Left	Congenital Dacryocystitis	Growth	Single	Streptococcus pneumoniae		N	S	N	S	N	S	R	N	N	N	N	N	S	N	S	N	N	N	N	N																						
3	38	M	761/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Escherichia coli		S	N	N	N	R	S	N	R	S	S	S	N	S	N	S	N	N	N	N	N																						
4	60	M	4018/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
5	21	F	685631/P	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
6	2	F	506824/IP	Unilateral	Right	Congenital Dacryocystitis	Growth	Single	Streptococcus pneumoniae		N	S	N	R	N	S	S	N	N	N	N	N	N	S	N	S	N	N	N	N	N																						
7	61	M	5053/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Mixed	Staphylococcus aureus	Candida albicans	S	N	S	R	R	S	N	N	R	N	N	S	S	N	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	S	S	S	S				
8	73	M	2094/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Enterococcus faecalis		N	N	N	S	S	N	N	N	N	N	N	N	S	R	S	N	S	N	N	N																						
9	58	M	6092/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
10	48	M	6038/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Mixed	Escherichia coli	Staphylococcus epidermidis	S	N	N	N	S	S	N	R	S	S	S	N	S	N	S	N	N	N	N	N	N	R	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	S	N	N	N
11	41	F	3887/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus		S	N	S	R	S	S	N	N	S	N	N	R	S	N	S	N	N	N	N	N	N																						
12	47	F	7541/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
13	3	M	505803/IP	Unilateral	Left	Congenital Dacryocystitis	No growth	-	-																																										
14	65	M	6945/OP	Bilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
					Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus		R	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N																						
15	54	F	7754/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae		S	N	N	N	R	S	N	R	S	S	S	N	R	N	S	N	N	N	N	N	N																						
16	27	F	7793/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
17	45	F	7540/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Mixed	Staphylococcus epidermidis	Klebsiella pneumoniae	S	N	S	R	S	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N	S	N	N	N	S	S	N	S	S	S	S	N	S	N	S	N	N	N	N	N
18	35	F	7374/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus		S	N	S	S	R	S	N	N	S	N	N	R	S	N	S	N	N	N	N	N	N																						
19	36	F	9245/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
20	41	F	9276/OP	Unilateral	Left	Acute Dacryocystitis	Growth	Single	Escherichia coli		S	N	N	N	R	S	N	S	S	S	S	N	S	N	S	N	N	N	N	N	N																						
21	36	F	7287/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
22	3	M	8165/OP	Unilateral	Left	Congenital Dacryocystitis	Growth	Single	Streptococcus pneumoniae		N	S	N	S	N	S	R	N	N	N	N	N	S	N	S	N	N	N	N	N	N																						
23	60	F	7637/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus		S	N	S	S	R	S	N	N	S	N	N	S	R	N	S	N	N	N	N	N	N																						
24	52	M	10174/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
25	68	F	11552/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus epidermidis		S	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																						
26	46	F	9614/OP	Unilateral	Left	Acute Dacryocystitis	Growth	Single	Staphylococcus aureus		S	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																						
27	35	M	8829/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Pseudomonas aeruginosa		S	N	N	N	R	N	N	S	S	N	S	N	N	N	N	S	N	N	N	N	N																						
28	35	F	10094/OP	Unilateral	Left	Acute Dacryocystitis	No growth	-	-																																										
29	43	F	17153/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
30	75	M	22983/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Mixed	Klebsiella pneumoniae	Staphylococcus aureus	S	N	N	N	R	S	N	R	S	S	S	N	S	N	S	N	N	N	N	N	N	S	N	S	S	R	R	N	N	S	N	N	S	S	N	S	N	N	N	N	N
31	53	F	21418/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
32	70	F	21427/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
33	1	F	21536/OP	Unilateral	Right	Congenital Dacryocystitis	No growth	-	-																																										
34	50	F	13197/OP	Bilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
					Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus epidermidis		R	N	S	S	S	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N																						
35	65	M	507155/IP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
36	63	F	24823/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Streptococcus pneumoniae		N	S	N	S	N	S	R	N	N	N	N	N	S	N	S	N	N	N	N	N	N																						
37	55	F	24872/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae		R	N	N	N	S	S	N	S	S	S	S	N	S	N	S	N	N	N	N	N	N																						
38	52	F	24280/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
39	65	F	24223/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus		R	N	S	S	R	S	N	N	R	N	N	R	R	N	S	N	N	N	N	N	N																						
40	71	F	23330/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
41	41	F	506312/IP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Escherichia coli		R	N	N	N	S	S	N	R	S	S	S	N	S	N	S	N	N	N	N	N	N																						
42	45	F	19169/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-																																										
43	60	F	507315/IP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Candida albicans		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	S	S	S	S																						
44	57	M	25331/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-																																										
45	52	F	25924/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae		S	N	N	N	R	R	N	S	S	S	S	N	S	N	R	N	N	N	N	N	N																						
46	57	F	25977/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Pseudomonas aeruginosa		S	N	N	N	S	N	N	R	S	N	S	N	N	N	N	S	N	N	N	N	N																						

47	19	F	507407/IP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
48	59	F	23090/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
49	4	M	23412/OP	Unilateral	Left	Congenital Dacryocystitis	Growth	Single	Staphylococcus aureus	.	S	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																					
50	45	F	16624/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
51	70	F	507301/IP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
52	46	F	21682/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Streptococcus pneumoniae	.	N	S	N	R	N	S	S	N	N	N	N	N	S	N	S	N	N	N	N	N	N																					
53	70	M	507207/IP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
54	36	F	17324/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus	.	S	N	S	S	S	S	N	N	S	N	N	R	S	N	R	N	N	N	N	N	N																					
55	45	F	505963/IP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae	.	S	N	N	N	R	S	N	S	S	S	S	N	S	N	S	N	N	N	N	N	N																					
56	50	F	15663/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
57	46	M	10750/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Enterococcus faecalis	.	N	N	N	S	R	N	N	N	N	N	N	N	S	S	S	N	S	N	N	N	N																					
58	55	M	20761/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
59	46	F	507059/IP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
60	65	F	507254/IP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Escherichia coli	.	R	N	N	N	R	S	N	R	S	S	S	N	R	N	S	N	N	N	N	N	N																					
61	4	F	9675/OP	Unilateral	Left	Congenital Dacryocystitis	Growth	Single	Staphylococcus epidermidis	.	S	N	S	S	S	S	N	N	S	N	N	R	S	N	S	N	N	N	N	N	N																					
62	67	M	24937/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
63	75	M	28185/OP	Unilateral	Right	Acute Dacryocystitis	Growth	Single	Staphylococcus aureus	.	R	N	S	S	S	S	N	N	R	N	N	R	S	N	S	N	N	N	N	N	N																					
64	59	F	22685/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
65	55	F	14179/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
66	62	F	26929/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus	.	S	N	S	R	R	S	N	N	S	N	N	R	R	N	S	N	N	N	N	N	N																					
67	48	F	29472/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
68	50	F	27216/OP	Unilateral	Right	Acute Dacryocystitis	Growth	Single	Staphylococcus epidermidis	.	S	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																					
69	78	F	25870/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
70	62	M	507819/IP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
71	45	M	39972/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Mixed	Pseudomonas aeruginosa	Staphylococcus epidermidis	R	N	N	N	R	N	N	S	S	N	S	N	N	N	N	S	N	N	N	N	N	S	N	S	S	S	S	N	N	R	N	N	R	S	N	S	N	N	N	N	N	N
72	50	M	22374/OP	Unilateral	Right	Acute Dacryocystitis	No growth	-	-	.																																										
73	6	F	6495/OP	Unilateral	Right	Congenital Dacryocystitis	Growth	Single	Pseudomonas aeruginosa	.	S	N	N	N	R	N	N	R	S	N	S	N	N	N	N	S	N	N	N	N	N																					
74	40	F	27881/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
75	78	F	31217/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae	.	S	N	N	N	R	S	N	S	S	S	S	N	S	N	S	N	N	N	N	N	N																					
76	56	F	507631/IP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
77	78	M	508503/IP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus epidermidis	.	R	N	S	R	S	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																					
78	2	M	30917/OP	Unilateral	Left	Congenital Dacryocystitis	No growth	-	-	.																																										
79	57	F	27958/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Streptococcus pneumoniae	.	N	S	N	S	N	S	R	N	N	N	N	N	S	N	S	N	N	N	N	N	N																					
80	2	M	507519/IP	Unilateral	Right	Congenital Dacryocystitis	No growth	-	-	.																																										
81	36	M	12690/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
82	45	F	28127/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus	.	R	N	S	S	S	S	N	N	R	N	N	S	S	N	R	N	N	N	N	N	N																					
83	59	F	27768/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
84	56	F	29225/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Pseudomonas aeruginosa	.	S	N	N	N	S	N	N	R	S	N	S	N	N	N	N	S	N	N	N	N	N																					
85	46	F	10637/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Staphylococcus epidermidis	.	S	N	S	S	S	S	N	N	S	N	N	R	S	N	S	N	N	N	N	N	N																					
86	65	M	28510/OP	Unilateral	Right	Chronic Dacryocystitis	No growth	-	-	.																																										
87	3	M	509217/IP	Unilateral	Left	Congenital Dacryocystitis	No growth	-	-	.																																										
88	46	F	29287/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae	.	R	N	N	N	S	S	N	R	S	S	S	N	S	N	S	N	N	N	N	N	N																					
89	46	F	29472/OP	Unilateral	Left	Acute Dacryocystitis	Growth	Single	Staphylococcus aureus	.	S	N	S	R	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																					
90	55	F	13201/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
91	58	M	32415/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Escherichia coli	.	S	N	N	N	R	R	N	S	S	S	S	N	S	N	S	N	N	N	N	N	N																					
92	6	F	508946/IP	Unilateral	Left	Congenital Dacryocystitis	Growth	Single	Staphylococcus aureus	.	R	N	S	R	S	S	N	N	R	N	N	R	S	N	S	N	N	N	N	N	N																					
93	54	M	24245/OP	Unilateral	Left	Acute Dacryocystitis	Growth	Single	Staphylococcus epidermidis	.	S	N	S	S	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																					
94	45	M	14418/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
95	50	F	15181/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Pseudomonas aeruginosa	.	S	N	N	N	R	N	N	S	S	N	S	N	N	N	N	S	N	N	N	N	N																					
96	62	F	509336/IP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
97	55	F	29213/OP	Unilateral	Left	Chronic Dacryocystitis	No growth	-	-	.																																										
98	64	F	15326/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Klebsiella pneumoniae	.	R	N	N	N	S	S	N	R	S	S	S	N	R	N	S	N	N	N	N	N	N																					
99	51	M	24565/OP	Unilateral	Right	Chronic Dacryocystitis	Growth	Single	Staphylococcus aureus	.	R	N	S	R	R	S	N	N	S	N	N	S	S	N	S	N	N	N	N	N	N																					
100	45	F	29419/OP	Unilateral	Left	Chronic Dacryocystitis	Growth	Single	Escherichia coli	.	S	N	N	N	R	S	N	S	S	S	S	N	S	N	S	N	N	N	N	N	N																					

KEY TO MASTER CHART

S	-	Sensitive
R	-	Resistant
N	-	Not tested
ORG 1	-	Organism 1
ORG-2	-	Organism 2
AK	-	Amikacin
Ox	-	Oxacillin
Cx	-	Cefoxitin
Ery	-	Erythromycin
Cip	-	Ciprofloxacin
Gati	-	Gatifloxacin
TMP-SMX	-	Trimethoprim-Sulfamethoxazole
Gen	-	Gentamicin
Tob	-	Tobramycin
CTX	-	Cefotaxime
CAZ	-	Ceftazidime
Pen	-	Penicillin
Tetra	-	Tetracycline
Amp	-	Ampicillin
Chlor	-	Chloramphenicol
PT	-	Piperacillin-tazobactam
Van	-	Vancomycin
Flu	-	Fluconazole
Itra	-	Itraconazole
Ampho	-	Amphotericin B
Vori	-	Voriconazole
M	-	Male
F	-	Female

BIBLIOGRAPHY

1. Sihota R, Tandon R. Parson's Diseases of the Eye.22nd edition. New Delhi: Elsevier;2015. P-475-78.

2. KhevnaPatel, RenuMagdum, SarikaSethia, Abhay Lune, AtreyeePradhan, Misra RN. A clinico-bacteriological study of chronic dacryocystitis. Sudanese J Ophthalmol.2014;6:1-5.

3. Shwetha BA, VittalNayakI. The bacteriological study of chronic dacryocystitis. IOSR Journal of Dental and Medical sciences.2014;13:30-36.

4. MyrenYanoff, Jay S. Duker. The lacrimal drainage system. In: Ophthalmology, 2nd edition. Mosby publication, P.761-769.

5. JithendraKandati, GudalaKiran Kumar, GauravaramAvanish, MadhavuluBuchineni, Rama Mohan Pathapati, PasupuletiSrinivas. Microbial surveillance of acute and chronic dacryocystitis in a tertiary care hospital.Journal of Evolution of Medical and Dental Sciences 2015; 4(3): 408-415.

6. Iliff NT. Infections of the lacrimal drainage system: Peopse JS, Holland GN, WilhelmusKR . Ocular Infection and Immunity. Mosby: St Louis, MO; 1996. P- 1346–55.

7. Nema HV, NitinNema. Text book of Ophthalmology. 6thedition. New Delhi: Jitendar P Vij;2012. P.441-43.

8. Coden DJ. Fungal dacryocystitis.Ophthalmology. 1993;100(2): 150

9. Arrington E.Jr.History of ophthalmology,NewYork, M.D.Publication 1959.

10. John J. woog. The incidence of symptomatic acquired lacrimal outflow obstruction among residents of olmsted county, minnesota, 1976-2000 (an american ophthalmological society thesis) trans am ophthalmol soc 2007;105:649-666

11. Hirschberg J. The History of Ophthalmology.Vol 1.Blodi FC, trans. Bonn, Germany: JP Wayenborgh; 1982:242.

12. Duke Elder S, Cook C. Diseases of the lacrimal passages. In: Duke-Elder S. Eds.System of Ophthalmology, Vol XIII, Part II. St. Louis, CV Mosby 1974: 675–724.

13. Codere F, Anderson RL. Bilateral Candida albicans dacryocystitis with facial cellulitis. Can J Ophthalmol. 1982; 17: 176.

14. Ramesh Murthy MS. Dacryocystitis.Kerala Journal of Ophthalmology Vol. XXIII, No.1, Mar. 2011:66-71.

15. Babar T, Masud M, Saeed N, Khan M. An analysis of patients with chronic dacryocystitis. Journal of Post Graduate Medical Institute. 2004; 18(3): 424-31.

16. Snell Richard S, Michael A. Clinical Anatomy of the Eye.2nd edition.Malaysia:Blackwell; 2009.P.114-123.

17. Nischal K, Pearson A. Jack J Kanski Brad Bowling Clinical Ophthalmology. 7th edition.China: Elsevier;2011. P-66-69.

18. VasudevAnand Rao. Text book of diseases of the eye. 3rd edition. Chennai: All India Publishers & Distributors;1999. P.41-48.

19. Dennis L. Kasper, Stephen L. Hauser,J. Larry Jameson, Anthony S. Fauci, Dan L. Longo, Joseph Loscalzo. Harrison's Principles of Internal medicine. 19thedition. New York :McGraw hill education;P-199-200.

20. Gupta AK, Usha K Raina, Aloke Gupta. Text book of Ophthalmology. New Delhi: BL Churchill Livingstone ;1998. P-274-76.

21. Khurana AK. Comprehensive Ophthalmology.4th edition. New Delhi: New Age International (P) Ltd;2007. P. 369-372.

22. Gerald L.Mandell John E.Bennett Raphael Dolin.Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 7thedition. Philadelphia: Churchill Livingstone Elsevier;2010. P 1569-71.

23. Pinar-Sueiro S, Sota M, Lerchundi T, Gibelalde A, Berasategui B, Vilar B, Hernandez J. Dacryocystitis: Systematic Approach to Diagnosis and Therapy. Curr Infect Dis Rep. 2012; 14:137-46

24. Suestevens. Lacrimal syringing.Community Eye Health. 2009 Jun; 22(70): 31.

25. Cuthbertson FM , Webber S. Assessment of functional nasolacrimal duct obstruction a survey of ophthalmologists in the southwest Eye (2004) 18, 20–23

26. Michael J Wearne, John Pitts, John Frank, Geoffrey E Rose. Comparison of dacryocystography and lacrimal scintigraphy in the

diagnosis of functional nasolacrimal duct obstruction. Br J Ophthalmol. 1999;83:1032–1035

27. Scott E Henney, Mike J Brookes, Kevin Clifford, AnirvanBanerjee.Dacryocystitis presenting as post-septal cellulitis: a case report. Journal of Medical Case Reports 2007, 1:77

28. Brooks Geo. F, Carroll Karen C, Butel Janet S, Morse Stephen A, Mietzner Timothy A.Jawetz, Melnick, &Adelberg's Medical Microbiology. 26th edition.NewYork: McGraw Hill;2013.P. 165-72.

29. Artikapil. Ananthanarayan and Paniker's Text book of Microbiology.9th edition.Hyderabad:Universities Press;2013.P. 621-23.

30. Patricia M. Tille. Bailey and Scott's Diagnostic Microbiology.13thedition.Missouri:Mosby Elsevier:2014. P. 910-18.

31. Syed Ali RazaRizvi, MeherRizvi, SnehalDhanrajRaut, Yogesh Gupta and PuneetMaheshwari.Etiology and Antimicrobial Sensitivity Pattern in Acute and Chronic Dacryocystitis.Int.J.Curr.Microbiol.App.Sci.2015 Special Issue-1: 269-80.

32. Sarkar I, Choudhury SK, Bandyopadhyay M Sarkar K, BiswasJ. A clinicobacteriological profile of Chronic Dacryocystitis in Rural India.International Journal of Health Sciences & Research. 2015; 5(7):82-87.

33. Eshraghi B, Abdi P, Akbari M, Fard MA. Microbiologic spectrum of acute and chronic dacryocystitis.Int J Ophthalmol 2014; 7 (5):864-67.

34. Pradeep A.V., Satish S. Patil, S.V. Koti, ArunKumar J.S., Santosh S. Garag, Jyotirmay S. Hegde.Clinico -Bacteriological Study of Chronic Dacryocystitis Cases in Northern Karnataka, India. Journal of Clinical and Diagnostic Research.2013;7(11):2502-04.

35. Prakash R, GirishBabuRJ,Nagaraj ER, Prasanth HV, Jeyashree S. A Bacteriological study of Dacryocystitis.Journal of Clinical and Diagnostic Research.2012;6(4):652-55.

36. Shah C P, Santani D. A comparative bacteriological profile and antibiogram of Dacryocystitis.Nepal J Ophthalmol. 2011;3(6):134-39.

37. Chaudhary M, Bhattarai A, Adhikari SK, Bhatta DR. Bacteriology and antimicrobial susceptibility of adult chronic dacryocystitis. Nep J Oph.2010;2(4):105-13.

38. Bharathi MJ, Ramakrishnan R, ManekshaV,Shivakumar C , Nithya V, Mittal S. Comparative bacteriology of acute and chronic dacryocystitis. Eye.2008; 22:953-60.

39. Mandal R, Banerjee AR, Biswas MC, Mondal A, Kundu PK, Sasmal NK. Clinicobateriological study of chronic Dacryocystitis in adults. J Indian Med Assoc.2008;106(5): 296-8.

40. BhavnaRaina, SudhirBhagotra.Clinicobacteriological Significance in Congenital Dacryocystitis.JK Science.2010;12(4):187-90.

41. AndreasKuchar, Julius Lukas, Franz Josef Steinkogler. Bacteriology and antibiotic therapy in congenital nasolacrimal duct obstruction.ActaOphthalmol.Scand. 2000; 78: 694–698.

42. Collee JG, Fraser AG, Marimion BP, Simmons A.Mackie and McCartney Practical Medical Microbiology.14th edition.Delhi:Churchill Livingstone Elsevier;2014. P.131-49.

43. Monica Cheesbrough. District Laboratory Practice in Tropical Countries.Part 2. 2nd edition. New York: Cambridge;2006. P-157-87.

44. Performance standards for Antimicrobial Susceptibility Testing; Twenty fourth Informational supplement.CLSI document M100-S24.

45. NteimamJonathan.Screening for Extended-Spectrum Beta-Lactamase-Producing Pathogenic Enterobacteria in District General Hospitals. J. Clin. Microbiol. 2005;43(3):1488-90.

46. Patricia M. Tille. Bailey and Scott's Diagnostic Microbiology.13th edition.Missouri:Mosby Elsevier:2014. P. 168-92.

47. Fran Fisher, Norma B Cook. Fundamentals of Diagnostic Mycology.Philadelphia:Saunders;1998. P. 196-211.

48. Washington Winn, Stephen D Allen, Willium M Janda, Elmer W Koneman, Gary W Procop, Paul C Schreckenberger, Gail L Woods. Koneman's Color Atlas and Textbook of Diagnostic Microbiology.6th edition.United States of America:LippincottWilliums& Wilkins;2006. P.1216-24.

49. JagdishChander.Text book of Medical Mycology. 3rd edition.New Delhi: Mehta publishers;2012. P.508-13.

50. Clinical and Laboratory Standards Institute(CLSI). Method for antifungal disc diffusion susceptibility testing of yeasts.Approved standard – M 44A.

51. Clinical and Laboratory Standards Institute(CLSI).2008. Reference method for Broth dilution antifungal susceptibility of yeasts. Approved standard – Third edition M27-A3.

52. Suharshi Gupta, Surbhi Gupta, Bella Mahajan.Bacteriology of Acquired Dacryocystitis In A TertiaryCare Hospital of North India. JK Science. 2014;16(3): 110-113.

53. JyotiBhuyan, Subhajit Das, Bhuyan SK. Clinicobacteriological and histopathological study in chronic dacryocystitis. Medpulse-International Medical Journal. 2014;1(3): 82-85.

54. Delia A, Ganesh C, Battacharjee K, DasD,Gogoi U. Bacteriology of Chronic Dacryocystitis in Adult Population of Northeast India. Orbit.2008; 27(4):243-47.

55. Chaudhry IA, Shamsi FA, Al-Rashed W. Bacteriology of chronic dacryocystitis in a tertiary eye care center. Ophthal Plast Reconstr Surg.2005;21(3):207-10.

56. Badhu B, Dulal S, Kumar S, Thakur SK, Sood A, Das H.Epidemiology of chronic dacryocystitis and success rate of external dacryocystorhinostomy in Nepal. Orbit 2005;24(2):79-82.

57. GillilandG.Agarwal S, Agarwal A, Apple DJ, BurattoL, Alio JL, Pandey SK. Text book of Ophthalmology. 1st edition. New Delhi: Jaypee brothers Medical Publishers;2002. 705-12.

58. Madhusudhan, YantiMuslikan, Nabilah Ismail, AdilHussein. Microbiological aetiology of acute dacryocystitis in hospital UniversitiSains Malaysia, Kelantan Malaysia. Journal of Acute Disease.2012; 31-34.

59. Kebede A, Adamu Y, Bejiga A. Bacteriological study of dacryocystitis among patients attending in Menelik II Hospital, Addis Ababa, Ethiopia. Ethiop Med J. 2010;48(1):29-33.

60. Mills,David M, Bodman, Marc G, Meyer, Dale R, Morton AD. The microbiologic spectrum of dacryocystitis: a national study of acute versus chronic infection.Ophthal Plast Reconstr Surg. 2007;23(4): 302-06.

61. Hartikainen J, Lehtonen O, MattiSaari K. Bacteriology of lacrimal duct obstruction in adults. British Journal of Ophthalmology. 1997;81:37-40.

62. Bareja U, Ghosh S. Clinicobacterial correlates of congenital dacryocystitis. Indian J Ophthalmol.1990;38:66-69.

63. Usha K, Smitha S, Shah N, Lalitha P, KelkarR.Spectrum and the susceptibilities of microbial isolates in cases of congenital nasolacrimal duct obstruction.JAAPOS.2006;10(5):469-72.

64. Ali MJ, Motukupally SR, Joshi SD, Naik MN. The microbiological profile of lacrimal abscess: two decades of experience from a tertiary eye care center. *Journal of Ophthalmic Inflammation and Infection* 2013;3(1):57.

65. Sun X, Liang Q, Luo S, Wang Z, Li R, Jin X. Microbiological analysis of chronic dacryocystitis. Ophthalmic Physiol Opt. 2005;25(3):261-63.

66. Razavi ME, Ansari-Astaneh MR, Farzadnia M, Rahmaniyan H, Moghiman T. Bacteriological evaluation of adult dacryocystitis in Iran.Orbit.2010;29(5):286-90.

67. Briscoe D, Rubowitz A, AssiaEI.Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. Orbit.2005;24(2):95-8.

68. Assefa Y, MogesF, EndrisM, Zereay B, Amare B, BekeleD,TesfayeS, MuluABelyhunY.Bacteriological profile and drug susceptibility patterns in dacryocystitis patients attending Gondar University Teaching Hospital, Northwest Ethiopia.BMC Ophthalmology. 2015; 15:34:1-8.

69. Brook I, FraizierFH. The aerobic and anaerobic microbiology of dacryocystitis.Am J Ophthalmol. 1998;125(4): 552-4.

70. Obi E, Roy A, Bates V, Sandy C.Bilateral chronic fungal dacryocystitis caused by Candida dubliniensis in a neutropenic patient. J clinPathol. 2006;59(11):1194-5.